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JCS36 U.S. PTO
06/20/00**UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)**

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
480.75-2 (HV)Total Pages in this Submission
70**TO THE ASSISTANT COMMISSIONER FOR PATENTS****Box Patent Application**

Washington, D.C. 20231

Transmitted herewith for filing under 35 U.S.C. 111(a) and 37 C.F.R. 1.53(b) is a new utility patent application for an invention entitled:

**CRYPTOPAIN VACCINES, ANTIBODIES, PROTEINS, PEPTIDES, DNA AND RNA FOR
PROPHYLAXIS, TREATMENT AND DIAGNOSIS AND FOR DETECTION OF
CRYPTOSPORIDIUM SPECIES**

and invented by:

CAROLYN PETERSEN, ET AL.JCS36 U.S. PTO
09/598062

06/20/00

If a CONTINUATION APPLICATION, check appropriate box and supply the requisite information:

☐ Continuation ☒ Divisional ☐ Continuation-in-part (CIP) of prior application No.: 08/827,171

Which is a:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.:

Which is a:

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No.:

Enclosed are:

Application Elements

1. ☒ Filing fee as calculated and transmitted as described below
2. ☒ Specification having 59 pages and including the following:
 - a. ☒ Descriptive Title of the Invention
 - b. ☒ Cross References to Related Applications (if applicable)
 - c. ☒ Statement Regarding Federally-sponsored Research/Development (if applicable)
 - d. ☐ Reference to Microfiche Appendix (if applicable)
 - e. ☒ Background of the Invention
 - f. ☒ Brief Summary of the Invention
 - g. ☒ Brief Description of the Drawings (if drawings filed)
 - h. ☒ Detailed Description
 - i. ☒ Claim(s) as Classified Below
 - j. ☒ Abstract of the Disclosure

**UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)**

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70

Application Elements (Continued)

3. ☒ Drawing(s) *(when necessary as prescribed by 35 USC 113)*
a. ☒ Formal b. ☐ Informal Number of Sheets 9
4. ☒ Oath or Declaration
a. ☐ Newly executed *(original or copy)* ☐ Unexecuted
b. ☒ Copy from a prior application (37 CFR 1.63(d)) *(for continuation/divisional application only)*
c. ☒ With Power of Attorney ☐ Without Power of Attorney
d. ☐ DELETION OF INVENTOR(S)
Signed statement attached deleting inventor(s) named in the prior application,
see 37 C.F.R. 1.63(d)(2) and 1.33(b).
5. ☐ Incorporation By Reference *(usable if Box 4b is checked)*
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied
under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby
incorporated by reference therein.
6. ☐ Computer Program in Microfiche
7. ☒ Genetic Sequence Submission *(if applicable, all must be included)*
a. ☒ Paper Copy
b. ☐ Computer Readable Copy
c. ☒ Statement Verifying Identical Paper and Computer Readable Copy

Accompanying Application Parts

8. ☒ Assignment Papers *(cover sheet & documents)*
9. ☐ 37 CFR 3.73(b) Statement *(when there is an assignee)*
10. ☐ English Translation Document *(if applicable)*
11. ☐ Information Disclosure Statement/PTO-1449 ☐ Copies of IDS Citations
12. ☒ Preliminary Amendment
13. ☒ Acknowledgment postcard
14. ☒ Certificate of Mailing
☐ First Class ☒ Express Mail *(Specify Label No.):* EL521153147US

**UTILITY PATENT APPLICATION TRANSMITTAL
(Small Entity)**

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Docket No.
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Accompanying Application Parts (Continued)

15. ☐ Certified Copy of Priority Document(s) *(if foreign priority is claimed)*

16. ☒ Small Entity Statement(s) - Specify Number of Statements Submitted: 1

17. ☐ Additional Enclosures *(please identify below)*:

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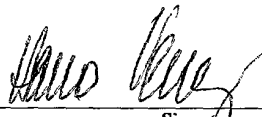
Fee Calculation and Transmittal

CLAIMS AS FILED

For	#Filed	#Allowed	#Extra	Rate	Fee
Total Claims	21	- 20 =	1	x \$9.00	\$9.00
Indep. Claims	3	- 3 =	0	x \$39.00	\$0.00
Multiple Dependent Claims (check if applicable) <input type="checkbox"/>					\$0.00
BASIC FEE					\$345.00
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Dated: JUNE 20, 2000


Signature
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Applicant or Patentee: CAROLYN PETERSEN AND JIN-XING HUANG Atty. Docket No.: 480.75-1 (HY)
 Serial or Patent No.: UNASSIGNED Date Filed or Issued: MARCH 27, 1997
 For: CRYPTOPAIN VACCINES, ANTIBODIES, PROTEINS, PEPTIDES, DNA AND RNA FOR
PROPHYLAXIS, TREATMENT, DIAGNOSIS AND DETECTION OF *Cryptosporidium parvum*

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
[37 C.F.R. 1.9(f) and 1.27(d)] - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: THE REGENTS OF UNIVERSITY OF CALIFORNIA
 ADDRESS OF ORGANIZATION: 300 LAKESIDE DRIVE, 22ND FLOOR, OAKLAND, CA 94612-3550

TYPE OF ORGANIZATION

- ☒ UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
☒ TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c) (3))
☒ NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA
 (NAME OF STATE CALIFORNIA)
 (CITATION OF STATUTE _____)
☐ WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 USC 501(a) and 501(c) (3)) IF LOCATED IN THE UNITED STATES OF AMERICA
☐ WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA
 (NAME OF STATE _____)
 (CITATION OF STATUTE _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 C.F.R. 1.9(e) for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled CRYPTOPAIN VACCINES, ANTIBODIES, PROTEINS, PEPTIDES, DNA AND RNA FOR PROPHYLAXIS, TREATMENT, DIAGNOSIS AND DETECTION OF *Cryptosporidium parvum* by inventors CAROLYN PETERSEN AND JIN-XING HUANG as described in:

- ☒ the specification filed herewith
☐ application serial no. _____, filed _____
☐ patent no. _____, issued _____

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 C.F.R. 1.9(d) or by any concern which would not qualify as a small business concern under 37 C.F.R. 1.9(d) or a nonprofit organization under 37 C.F.R. 1.9(e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 C.F.R. 1.27)

NAME NOT APPLICABLE
 ADDRESS _____
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING LINDA S. STEVENSON
 TITLE IN ORGANIZATION SENIOR PROSECUTION ANALYST, OFFICE OF TECHNOLOGY
 ADDRESS OF PERSON SIGNING 300 LAKESIDE DRIVE, 22ND FLOOR, OAKLAND, CA 94612-3550

SIGNATURE _____

DATE March 27, 1997

JC836 U.S. PTO
 09/598062
 06/20/00

480.75-2

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of)
Carolyn Petersen, et al.) Group Art No.: N/A
Serial No.: N/A) Examiner: N/A
Filed: June 16, 2000)

For: Cryptopain Vaccines, Antibodies, Proteins,
Peptides, DNA and RNA for Prophylaxis, Treatment
and Diagnosis and for Detection of *Cryptosporidium*
Species

BOX PATENT APPLICATION
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited
with the United States Postal Service as Express Mail label no.
EL521153147US addressed to Commissioner of Patents and Trademarks,
Washington, D.C. 20231 on June 20, 2000.


Hana Verny (Reg No. 30,518)

PRELIMINARY AMENDMENT

This Preliminary Amendment is filed concurrently with the
filing of a Divisional Application under 37 C.F.R. 1.53(b).

In the Specification

Page 1, first paragraph, please amend the first paragraph as
follows:

--This application is a Divisional application of
Serial No. 08/827,171, filed March 27, 1997, allowed,
which is--.

In the Claims

Cancel claims 1-18. Examine new claims 19-39.

19. (New) An antibody binding to a *Cryptosporidium* antigen cryptopain comprising an amino acid sequence SEQ ID NO: 4 or a fragment thereof.

20. (New) The antibody of Claim 19 binding to the fragment of SEQ ID NO: 4, said fragment identified by an amino acid sequence SEQ ID NO: 5.

21. (New) The antibody of Claim 19 binding to the fragment of SEQ ID NO: 4, said fragment identified by an amino acid sequence SEQ ID NO: 6.

22. (New) The antibody of Claim 19, wherein the antibody is monoclonal or polyclonal.

23. (New) The antibody of claim 19 detecting a presence of *Cryptosporidium* by formation of an antibody-antigen complex.

24. (New) The antibody of claim 22 wherein the antibody is polyclonal.

25. (New) The antibody of claim 22 wherein the antibody is polyclonal.

26. (New) A method of treatment of *Cryptosporidium* infections comprising administering to a subject in need of such treatment an anti-*Cryptosporidium* antibody binding to a protein comprising a sequence SEQ ID NO: 4 or a fragment thereof, an anti-*Cryptosporidium* vaccine comprising a *Cryptosporidium* antigen cryptopain comprising a sequence SEQ ID NO: 4 or a fragment thereof, or a DNA or RNA vaccine comprising a DNA sequence identified as SEQ ID NO: 1 or a fragment thereof.

27. (New) The method of claim 26 comprising administration of the anti-*Cryptosporidium* antibody.

28. (New) The method of claim 26 comprising administration of anti-*Cryptosporidium* vaccine comprising the *Cryptosporidium* antigen cryptopain comprising the amino acid sequence SEQ ID NO: 4 or the fragment thereof identified by the amino acid sequence SEQ ID NO: 5 or SEQ ID NO: 6.

29. (New) The method of claim 28 wherein the vaccine comprises the amino acid sequence SEQ ID NO: 4.

30. (New) The method of claim 28 wherein the vaccine comprises the fragment identified as SEQ ID NO: 5.

31. (New) The method of claim 28 wherein the vaccine comprises the fragment identified as SEQ ID NO: 6.

32. (New) The method of claim 26 wherein the vaccine comprises the DNA sequence identified as SEQ ID NO: 1 or the fragment thereof identified as SEQ ID NO: 2 or SEQ ID NO: 3.

33. (New) The method of claim 31 wherein the vaccine comprises the DNA sequence identified as SEQ ID NO: 1.

34. (New) The method of claim 31 wherein the vaccine comprises the DNA fragment identified as SEQ ID NO: 2.

35. (New) The method of claim 31 wherein the vaccine comprises the DNA fragment identified as SEQ ID NO: 3.

36. (New) A method of diagnosing *Cryptosporidium* infection, comprising steps:

(a) contacting a sample of a body specimen, fluid or tissue obtained from a subject, with an anti-*Cryptosporidium*

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antibody having specificity for an antigen identified by an amino acid sequence SEQ ID NO: 4 or a fragment thereof identified by the amino acid sequence SEQ ID NO: 5 or SEQ ID NO: 6; and

(b) detecting a formation of an antibody/antigen complex in the sample.

37. (New) The method of claim 36 wherein the antibody binds to the antigen identified by the amino acid sequence SEQ ID NO: 4.

38. (New) The method of claim 35 wherein the antibody binds to the antigen identified by the amino acid sequence SEQ ID NO: 5.

39. (New) The method of claim 35 wherein the antibody binds to the antigen identified by the amino acid sequence SEQ ID NO: 6.

REMARKS

This Preliminary Amendment is submitted concurrently with the filing of a Divisional application. It is believed that the newly submitted claims are in better condition for examination.

Respectfully submitted,

Date: June 20, 2000



Hana VERNY (Reg. No. 30,518)
Attorney of Record

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Atty Dkt: 480.75-2 (HV)

CRYPTOPAIN VACCINES, ANTIBODIES, PROTEINS, PEPTIDES, DNA AND
RNA FOR PROPHYLAXIS, TREATMENT AND DIAGNOSIS AND FOR
5 DETECTION OF *Cryptosporidium* SPECIES

This application is a based on the provisional application Ser. No. 60/014233 filed on March 27, 1996.

This invention was developed partially with U.S. Government support under National Institutes of Health Grant
10 No U01-AI35123. The U.S. Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

Field of the Invention

This invention concerns vaccines, antibodies, proteins,
15 DNAs and RNAs for diagnosis, prophylaxis and treatment of *Cryptosporidium* species infections and for detection of *Cryptosporidium* species. In particular, this invention concerns *Cryptosporidium* species antigen comprised of a protein, as well as polyclonal and monoclonal antibodies
20 directed against the antigen, DNAs and RNA encoding the *Cryptosporidium* species antigen and fragments and analogs thereof, and methods for production of recombinant or fusion proteins. This invention also concerns methods for diagnosis, prophylaxis, treatment of *Cryptosporidium* infections and
25 detection of *Cryptosporidium* species.

BACKGROUND AND RELATED DISCLOSURES

The genus *Cryptosporidium* consists of Apicomplexan parasites that invade and develop within epithelial cells of the gastrointestinal, hepatobiliary and respiratory tracts of
30 a wide variety of vertebrates including reptiles, birds and mammals. *Cryptosporidium* was recognized as a cause of animal disease for several decades before the first cases of human cryptosporidiosis were reported in 1976. However, it was not

until 1982 that the magnitude of disease caused by this parasite in both AIDS patients and immunocompetent hosts began to be appreciated. Subsequently, *Cryptosporidium* has been found to be one of the most common causes of human diarrhea worldwide, and to be an increasingly recognized cause of diarrhea in children, animal care workers, and travelers. (*Cryptosporidium* and *Cryptosporidiosis* in Humans, Ed. Fayer, R., CRC Press, Boca Raton (1997)).

Large waterborne outbreaks of cryptosporidiosis caused by contaminated municipal water supplies in the US or in the UK have been noted in the last ten years (*N. Engl. J. Med.*, 320:1372 (1989), and 33:161 (1994)). The most recent outbreak in Milwaukee in April 1993 involved 400,000 persons and led to the subsequent deaths of more than 100 immunocompromised persons. Like a number of other waterborne outbreaks, the Milwaukee outbreak appears to have been due to contamination from farm or abattoir run-off and specifically to cryptosporidiosis among cows/calves. Nosocomial transmission in hospitals from patients to staff, patient to patient, and contaminated ice to patients and staff have also been well documented (*J. Infect. Dis.*, 158:647 (1985)).

Waterborne and nosocomial spread uncovered a number of biological characteristics of oocysts. First, the infectious dose of a parasite is very low. The ID₅₀ for human volunteers with normal immune systems is 132 oocysts *N. Engl. J. Med.*, 332:855 (1995). Second, infected hosts, for example calves, excrete large numbers of oocysts, on the order of 10¹⁰/day. Third, the oocysts are fully sporulated and ready to infect when excreted. Fourth, the oocysts are environmentally hardy. They remain infectious in cool, moist areas for 3-4 months. They are not killed by chlorine levels achievable in drinking water. Fifth, the oocysts are quite small, 4-6 μ m, and are thus difficult to filter.

The clinical importance of cryptosporidiosis has increased markedly with the recognition of a life-threatening form of the disease in patients with immunodeficiency disorders such as AIDS, hypogammaglobulinaemia, and
5 chemotherapeutic immunosuppression. The prevalence of cryptosporidiosis in AIDS patients in the US is estimated to be 5-10% and in central Africa 40%. Immunodeficient patients may have fulminant cryptosporidial diarrhea that may persist until death, whereas the diarrhea of immunocompetent patients
10 is self-limited and rarely lasts more than 2-4 weeks. Cholera-like diarrhea is common in immunocompromised patients with reported losses of up to 17 liters per day. Hepatobiliary disease may result in severe abdominal pain and nausea. Removal of immunosuppression in chemotherapy patients
15 leads to resolution of the diarrhea. Some AIDS patients with cryptosporidiosis will be able to eliminate the parasite by induction of anti-retroviral therapy (Am. Intern. Med., 116:840 (1992)).

Among those who develop disease, a quarter have CD4
20 counts greater than 209, suggesting that the disease may occur relatively early in the course of HIV disease (Am. J. Epidemiol., 144:807 (1996)). Unfortunately, few details about the biology and molecular mediators of the disease process have been described and so far no effective therapy has been
25 discovered.

The infective forms of *Cryptosporidium*, called sporozoites and merozoites, appear to adhere to the host cell and release the contents of anterior organelles (rhoptries, micronemes or dense granules) during the invasion process
30 (Parasitol. Today, 8:28(1992)). Proteins involved in these events have in many instances been found to be the target of invasion blocking immunity *in vitro* and neutralization *in vivo* (Infect. Immun., 56:2538(1988)).

Active and passive immunization studies using malaria and *Toxoplasma* challenged or infected hosts have shown that certain secreted components of the apical complex organelles are the target of protective antibodies. In some cases, as
5 for example in the case of the circumsporozoite and merozoite surface proteins of malaria, these antigens are under development as vaccines.

While the actual interaction between *Cryptosporidium* and the host's immune system is poorly understood, it is known
10 that disruption of either the cellular or the humoral components can result in protracted cryptosporidiosis (Parasitol. Today, 8:24 (1992)). However, specific antibodies alone appear to be enough to neutralize the organism's infectivity. *In vitro* and *in vivo* observations indicate that
15 antibodies to *Cryptosporidium parvum* inhibit invasion and intracellular development leading to protection in challenge experiments, or amelioration of infection in established disease (Infect. Immun., 59:1172 (1991)).

One source of such antibodies is hyperimmune bovine
20 colostrum (HBC) collected from cows immunized with *Cryptosporidium* oocysts. Calves challenged with *Cryptosporidium* oocysts are protected from the development of disease by the administration of HBC (Infect. Immun., 61:4079 (1993)). Some immunocompromised AIDS patients infected with
25 *Cryptosporidium* have also responded to HBC with a reduction in or disappearance of the symptoms of the disease (Gastroenterology, 98:486 (1990)). Immunoglobulin from HBC (HBC Ig) has been found to inhibit the ability of the sporozoite to invade and/or develop intracellularly *in vitro*
30 and it has been used to immunoprecipitate at least 22 different surface radioiodinated proteins of *Cryptosporidium* sporozoites. Western blot analysis of proteins of whole oocysts which contain sporozoite, indicates that HBC

predominantly recognizes two proteins of sizes 250 Kd and >900 Kd (Infect. Immun., 61:4079 (1993)).

The use of HBC for human use is problematic. HBC produced using whole oocysts is batch dependent and this may lead to the development of passive immune preparations which are not uniform in immunogenicity and potency. This generates a problem when these immune preparation are to be administered to human patients as such non-uniformity may result in failure of protection. In addition, it would be desirable to allow preparation of large amounts of antigen expressed in heterologous systems than to purify oocyst.

Thus, there is a continuous need for immunogenic agents which are reasonably reproducible and have uniform and controllable immunogenicity and potency which agents would be useful for the immunotherapy of cryptosporidiosis in both uncompromised and immunocompromised subjects, such as AIDS patients, and would allow the prophylaxis and treatment of cryptosporidiosis.

Additionally, there is a need to have available methods for reproducible expression of specific target for *Cryptosporidium* antigen in large amounts, which antigen would provide a better immunogen. This approach requires that a specific *Cryptosporidium* antigen is cloned and identified as a potential candidate through its ability to elicit an antibody response that is immunoprotective. Before antibodies produced in this manner are tested in or administered to humans or animals, testing in *in vitro* assay of their inhibitory effect on invasion or intracellular development of the *Cryptosporidium* organism in cultured cells and *in vivo* studies would be desirable.

It is, therefore, a primary objective of this invention to provide *Cryptosporidium* cryptopain polyclonal or monoclonal antibodies and vaccines to be used for prophylaxis, treatment,

diagnosis and detections of cryptosporidiosis and to express a portion of the cryptopain sequence/locus to provide target protein antigens allowing production of recombinant anti-*Cryptosporidium* vaccines and passive immune products.

5 All patents, patent applications and publication cited herein are hereby incorporated by reference.

SUMMARY OF THE INVENTION

One aspect of this invention concerns vaccines, antigens, antibodies, proteins, DNAs and RNAs for prophylaxis, treatment
10 and detection or diagnosis of *Cryptosporidium* species or *Cryptosporidium* species infections.

Another aspect of this invention concerns a *Cryptosporidium* antigen protein comprising pre, pro, and mature enzyme sequences and their fragments.

15 Still another aspect of this invention concerns polyclonal or monoclonal antibodies directed against the *Cryptosporidium* antigen.

Still yet another aspect of this invention concerns a DNA and RNA encoding the *Cryptosporidium* antigen and fragments
20 thereof and the antigen pre, pro, and mature regions.

Another aspect of this invention concerns a polyclonal or monoclonal antibodies directed against invasive stages of *Cryptosporidial* species capable of preventing and ameliorating invasion of *Cryptosporidium* infection.

25 Still another aspect of this invention concerns a natural, synthetic or recombinant vaccine useful for active immunization of animals and humans against *Cryptosporidium* infection.

Still another aspect of this invention concerns a
30 natural, synthetic or recombinant protein useful for preparation of passive immune products for treatment of established infection.

Another aspect of this invention concerns a natural, synthetic or recombinant DNA vaccine capable of endogenous production of inhibitory amount of anti-*Cryptosporidium parvum* antibodies.

- 5 Another aspect of this invention concerns a natural, synthetic or recombinant RNA vaccine capable of endogenous development of inhibitory amount of anti-*Cryptosporidium parvum* antibodies.

- 10 Still another aspect of the invention concerns a method for use of a pre pro enzyme portion of the cysteine proteinase molecule as a competitive inhibitor of the action of the mature enzyme.

- 15 Still yet another aspect of the invention is the use of antigen, antibody, DNA or RNA to detect the presence of the cysteine proteinase or antibodies to cysteine proteinase, or DNA or RNA encoding the cysteine proteinase, for diagnosis in a human or animal host or detection in the environment.

- 20 Another aspect of this invention concerns the sequence of a 401 amino acid protein comprising a cathepsin L-like cysteine proteinase of MW 45 kDa present in sporozoites and merozoites, and its amino acid and size variants including a deduced mature 226 amino acid protein of MW 25 kDa.

- 25 Another aspect of this invention concerns the DNA sequence of 1203 nucleotides encoding the 45 kDa protein, the cathepsin-like cysteine proteinase, cryptopain, its nucleotide and size variants and its upstream regulatory elements.

- 30 Another aspect of this invention concerns the RNA sequence determined by the DNA sequence of cryptopain and its nucleotide and size variants including polyadenylation sequence.

Still yet another aspect of this invention concerns a group of cryptopain recombinant or expressed protein targets of polyclonal antibodies which inhibit *Cryptosporidium*

infection, invasion, or adhesion.

Another aspect of this invention concerns a method for prophylaxis and treatment of *Cryptosporidium* or *Cryptosporidium* infections using vaccines, antibodies, proteins, DNAs and RNAs of the invention.

Still yet another aspect of this invention concerns a method of prophylaxis, treatment, inhibition or retardation of a *Cryptosporidium* infection comprising administering to a subject in need of such treatment an amount of an anti-*Cryptosporidium* polyclonal or monoclonal antibodies prophylactically or therapeutically effective to provide immunity against infection or treatment for disease.

Still yet another aspect of this invention concerns a method of prophylaxis, treatment, retardation, or inhibition of *Cryptosporidium* infection comprising administering to a subject in need of such treatment a vaccine comprising the polypeptide of this invention or its DNA or RNA capable of endogenous stimulation of the production of inhibitory amount of anti-*Cryptosporidium* antibodies or protective cellular immune responses.

Still yet another aspect of this invention concerns a method for diagnosing *Cryptosporidium* infection of a subject, comprising steps:

(a) contacting a body specimen, fluid or tissue obtained from the subject with an anti-*Cryptosporidium* monoclonal or polyclonal antibody; and

(b) detecting the formation of antibody-antigen complex wherein the presence of the complex indicates the presence of a *Cryptosporidium* organism in the subject.

Still yet another aspect of this invention concerns a method for detecting anti-*Cryptosporidium* antibody in a subject, said method comprising steps:

(a) contacting a body specimen, fluid or tissue obtained from the subject with the cryptopain; and

(b) detecting a formation of antibody-antigen complex wherein the presence of the complex indicates the presence of
5 a *Cryptosporidium* antibody in the subject.

Still another aspect of this invention is a *Cryptosporidium* diagnostic or detection kit comprising anti-*Cryptosporidium* specific monoclonal and polyclonal antibodies or antigen according to the invention and a means
10 for detection of an antibody-antigen complex.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram showing the strategy model for developing a probe for the *Cryptosporidium* cysteine proteinase using consensus oligonucleotide primers for PCR amplification
15 of genomic DNA. The model is compared to previously published diagrams of the primary structure of cysteine proteinases from other organisms.

Figure 2 is the DNA sequence of cryptopain (SEQ ID NO: 1) comprising sequences encoding segments for the pre and pro regions (SEQ ID NO: 2), mature enzyme coding region (SEQ ID
20 NO: 3) and 3' and 5' flanking sequences.

Figure 3 is the protein sequence of cryptopain (SEQ ID NO: 4) comprising segments for the pre and pro regions (SEQ ID NO: 5) and for mature enzyme (SEQ ID NO: 6).

25 Figure 4 is an amino acid alignment showing marked amino acid similarities of cryptopain to other cathepsin-like cysteine proteinases (SEQ ID NOS: 4, 7 and 8).

Figure 5 shows a genomic Southern analysis of *Cryptosporidium* DNA using the cryptopain probe.

30 Figure 6 shows a Kyte Doolittle hydropathy plot indicating an N-terminal hydrophobic sequence consistent with membrane targeting and secretion of cryptopain.

Figure 7 are oligonucleotide sequences used to generate

DNA fragments of the cryptopain gene. Figure 7A1 is a degenerate primer based on the conserved cysteine (sense) and Figure 7A2 is a degenerate primer based on conserved arginine (antisense) of the *P. vinckei* cysteine proteinase gene. These
5 primers were used to amplify the 459 bp fragment of cryptopain from *C. parvum* DNA. Figure 7B shows primers used to directionally clone the entire *C. parvum* gene comprising pre, pro and mature protein encoding regions, to be expressed as a thioredoxin fusion protein. Figure 7B1 is the sense and
10 Figure 7B2 is the antisense oligonucleotide.

Figure 8 is a diagram of pTrxFus showing the directional cloning strategy.

Figure 9 is a Western blot of cryptopain expressed as a thioredoxin fusion protein and detected by anti-thioredoxin
15 antibody.

Figure 10 are graphs showing percentage of invasion/intracellular development of *Cryptosporidium parvum* sporozoites *in vitro* in MDCK cells in the presence of inhibitors of cysteine proteinases. Figure 10A is a
20 comparative graph of three cysteine proteinase inhibitors biotinylated fluoromethylketone (BPAFMK) (Figure 10B); trans-epoxysuccinyl-L-leucylamido-(4-guanidino) butane E64 (Figure 10C); and proprietary compound K-111 (Figure 10D). Figures 10B-10D show standard deviations.

25

DEFINITIONS

As used herein:

"Cryptopain" or "*Cryptosporidium* antigen" means a protein which is a cathepsin L-like cysteine proteinase having a function in invasion and infection of host cells by
30 *Cryptosporidium*. Cryptopain is represented by a protein containing 401 amino acids and is identified as SEQ ID NO: 4 (Figure 3) comprising a protein of MR 45 kDa. Homology to other cathepsin L-like cysteine proteinases seen in Figure 4

indicates that the mature active enzyme is cleaved after amino acid 175 one residue N-terminal to a conserved prolines and comprises a 25 kDa protein of 226 amino acids. Cryptopain also includes size and sequence variance proteins which
5 maintain the same function.

The "structure" or "structural characteristics" of cryptopain defines a protein, and DNA and RNA encoding the cryptopain protein and includes all structural variations, mutations and fragments exhibiting the same function.

10 The "functionality" or "functional characteristics" of cryptopain is defined by the action of the protein and structural variants described, such that infection and disease occurs.

"Inactive enzyme" means enzyme comprised either of mature enzyme regions and pro regions, or mature enzyme and pro and pre regions wherein the pro or pre pro regions are responsible for the mature enzyme nonfunctionality or for the inhibition of its function.
15

"Active enzyme" or "mature enzyme" means functional enzyme and is comprised of the mature region. Mature enzyme contains the catalytic active sites of the cysteine proteinase and typically begins with one residue N-terminal to a conserved proline.
20

"Pro" or "pro region" means the contiguous amino acid sequence which renders the mature enzyme inactive by its structural association with it.
25

"Pre" or "pre region" means the terminal amino acid sequence which is contiguous with the pro region and may contain a signal for trafficking movement of inactive enzyme in the cell.
30

"The gene" or "genes encoding cryptopain" means DNA encoding the cryptopain protein.

"Sporozoites or merozoites " mean any life stage which

may invade or develop in the host cells and any variant or mutant of said life stages.

"Antibodies" means proteins which structurally interact with the target antigen and are produced when the antigen is introduced into an animal, such that they stimulate the immune system. The term also includes antibodies produced *in vitro*, such as chimeras, or hybridoma cell cultures, as well as hybridomas or chimeric constructs introduced into a host to provide an *in vivo* antibody.

10 "Antibodies to cryptopain" means proteins which structurally interact with the target antigen cryptopain and inhibit invasion, infection or development of the sporozoites or merozoites in the host cell.

"Monoclonal antibodies" means the monovalent antibodies produced by an B cell fused to an immortalized cell producing specific antibody to cryptopain.

"Polyclonal antibodies" means antibodies directed at cryptopain which are not monovalent and are the products of multiple B cells in character.

20 "Cryptosporidium antigen" means a protein with or without carbohydrate attached thereto which defines a capacity of *Cryptosporidium* sporozoites and merozoites to infect and develop in host cells.

"Cryptopain DNA" means the sequence of 1203 polydeoxyribo nucleotides identified in SEQ ID NO: 1 (Figure 2) which encodes the amino acid sequence of *Cryptosporidium* antigen (SEQ ID NO: 4) and any variants, mutations and fragments thereof which correspond to or would detect genes encoding the antigen and includes specific PCR oligonucleotide primers for amplification of cryptopain sequences and fragments of sequence used as genetic probes for detection of cryptopain sequence. Also included is DNA inserted into host cells for the purpose of *in vivo* expression of target antigen in order

to stimulate the host immune system.

"Cryptopain RNA" means the sequence of 1203 nucleotides which encodes the protein sequence of cryptopain protein (SEQ ID NO: 4) (Figure 3) and any variants, mutations and fragments thereof including polyadenylation tail which correspond to or would detect genes encoding the antigen. RNA probes and RNA inserted into host cells for the purpose of *in vivo* expression of target antigen in order to stimulate the host immune system are included.

"Vaccine" means protein, recombinant protein, DNA or RNA from cryptopain which, upon introduction into a host, is able to provoke an immune response including but not limited to the production of antibodies, cytokines and other cellular responses.

"Detection" means establishing or providing evidence for the presence or prior presence of living or dead *Cryptosporidium* by detecting cryptopain protein, *Cryptosporidium* protein specific activity, DNA or RNA in the host, in a host tissue specimens, or in environmental samples including water, soil, food, etc.

"Diagnosis" means establishment of the presence or prior presence of *Cryptosporidium* infection or disease by using the cryptopain protein, *Cryptosporidium* protein specific activity, DNA or RNA as a component of a diagnostic assay according to the invention.

"Prevention or prophylaxis" means the immunization or vaccination of the host with a vaccine of the invention such that *Cryptosporidium* disease or infection does not occur.

"Treatment" means therapeutic use of any protein or antibody to inhibit *Cryptosporidium* infection in a host.

"Host" or "subject" means human, or animal including birds and cattle.

"Regulatory elements" means nucleotide sequences which

control the expression of genes they regulate, typically by interaction with other macromolecular species such as protein.

"Active immunity to infection" means ability of the organism to produce specific responses such as production of cytokines, lymphokines, antibodies or other substances, or cellular capacity to inhibit or retard infection in response to a contact with antigen.

"Passive immunity to infection" means the transfer to a host of the specific antibodies or other substances or cells capable of inhibiting or retarding infection.

"*Cryptosporidium* species" means any organism belonging to the genus *Cryptosporidium*, such as, for example, *Cryptosporidium parvum* or *Cryptosporidium muris*, but also includes currently less well characterized other organisms such as, for example, *Cyclospora* and similar organisms, such as *Eimeria*. *Cryptosporidium* species comprise Apicomplexan parasites which primarily invade cells of gastrointestinal tract and cause disease in a susceptible host.

"Recombinant vaccines" means DNA/RNA/protein segments propagated or expressed in foreign system. This includes all vaccines other than biologically derived vaccines.

"Biologically derived vaccines" means vaccines made from a protein or carbohydrate generated in the organism of origin.

DETAILED DESCRIPTION OF THE INVENTION

The current invention is based on findings that cryptopain, a cathepsin L-like cysteine proteinase, localized at the *Cryptosporidium* sporozoites surface or within its cell, is involved in *Cryptosporidium* infectivity and that such infectivity can be prevented by cryptopain inhibitors.

Cryptopain deduced amino acid sequence shows homology to other cathepsin L-like cysteine proteinases indicating that the mature active enzyme is a 25 kDa protein of 225 amino acids. Cryptopain DNA has been isolated, purified, sequenced

and recombinantly produced. Cryptopain fusion protein in which the fusion partner is thioredoxin has also been recombinantly produced.

Due to its unique biological activity, cryptopain may be advantageously used for prophylactic, therapeutic, diagnostic and detection purposes.

This invention, therefore, relates to isolated native and recombinantly produced cryptopain; cryptopain amino acid, DNA and RNA sequences; and to vaccines, antibodies, proteins and synthetic proteins, DNAs and RNAs useful for prophylaxis, treatment, diagnosis and detection of infections caused by any *Cryptosporidium* organism or any organism belonging to *Cryptosporidium* species.

More specifically, the invention concerns identification and cryptopain of a *Cryptosporidium* antigen, comprised of a protein or polypeptide, identification of DNA of the *Cryptosporidium* antigen gene within the locus, sequencing DNA encoding the *Cryptosporidium* antigen, expressing portions of the locus encoding the *Cryptosporidium* antigen and using the expressed antigens for preparation of vaccines or for preparation of polyclonal or monoclonal antibodies.

I. Cryptopain - *Cryptosporidium* Parvum Antigen

Cryptopain is cathepsin L-like cysteine proteinase. It is structurally and functionally similar to other cysteine proteinases, represented, for example, by *Carica* papain and *Plasmodium vinckei* cysteine proteinase, and its activity is inhibited by group of cysteine proteinase specific inhibitors.

A. Cysteine Proteinases - Their Function, Structure and Inhibition

There are four major classes of proteinases for which the catalytic mechanism has been defined. These proteinases are designated cysteine, aspartic, metallo and serine proteinases. The major mammalian cysteine proteinases are the lysosomal

cysteine proteinases, cathepsins B, H and L proteinases and the cytoplasmic calpains. Mammalian cysteine proteinases B and L are also active at neutral pH, and are found outside the cell and may function in the degradation of extracellular proteins. The sequences of the protozoan cysteine proteinases identified to date show that they are more closely related to cathepsin L than to cathepsin B. Cysteine proteinases essentially contain amino acids cysteine, histidine and asparagine which are important for the action of the proteinases. The sulfonium ion of the cysteine provides the nucleophilic attack on the carbonyl group of the targeted peptide bond in order to effect hydrolysis of the bond.

Calpains and cathepsins are generally distinguished from each other by their cellular locations and by their inhibition profile. For example, cathepsins, but not calpains, are inhibited by the peptidyl diazomethane and peptidyl fluoromethylketone inhibitors Z-phe-ala-CHN₂ (diazomethane) and Z-phe-ala-FMK (fluoromethylketone). Both lysosomal cysteine proteinases and calpains are inhibited by the class-specific inhibitor E64 and the more general inhibitor leupeptin.

Peptide inhibitors have been used to determine the peptide bond specificity of proteinases. The specificity of the inhibitor is determined by the amino acid residues, for example, phe-ala residues, which bind in the pocket formed by the active sites of the enzyme. Peptide inhibitors only bind to active enzyme, i.e. enzyme which has a conformationally correct enzyme pocket. Peptide inhibitors are useful for detection of the presence of specific types of cysteine proteinases in living systems as they may allow the localization or detection of enzymatic activity in the absence of isolation and purification of the enzyme with the subsequent development of antibody probes. Since isolation of

active enzyme by biochemical techniques requires large amounts of material and the isolated enzyme is often not stable, use of peptide inhibitors instead is very advantageous.

Proteinase inhibitors are a new type of agent for
5 treatment of protozoan infection. Cloning of genes for
selected proteinases, expression of the proteinases, and
molecular modeling of the proteinases are techniques which
have facilitated the development of cysteine proteinases
inhibitors specific for a given enzyme, such as for example,
10 falcipain of *P. falciparum*. In addition, the differences
between mammalian and protozoan cysteine proteinases and
between cysteine proteinases of specific protozoa allow
development of detection techniques for the organism based on
the acting of the enzyme, DNA, RNA and antibodies.

15 B. Cryptopain Gene Cloning, Sequencing and Genomic
Southern Analysis

In order to provide consistently the same antigen for
production of antibodies or vaccines, and for recombinant
production of fusion proteins and other agents useful for
20 prophylactic therapeutic and diagnostic purposes, cryptopain
was cloned, sequenced and genomic Southern analysis was
performed to determine whether there was one or more cysteine
proteinase similar to cryptopain.

Degenerative oligonucleotides were synthesized from the
25 sequences encoding the active sites of papain like cysteine
proteinases centered around the active site cysteine and
histidine as seen in Figure 1 and around the active site
arginine described in Example 2. In Figure 1, the primary
structures of cysteine proteinases for *L. mexicana*, *T. brucei*,
30 and human cathepsin-L are compared to the primary structure of
C. parvum cryptopain. The diagram in Figure 1 shows the
conserved cysteine and histidine residues involved in the
active site, and the cysteine residues apparently involved in

disulfide bridges. For cryptopain, the conserved cysteine is C-24, the conserved histidine is H-164. The proposed disulfide bridges are 21-65, 56-103 and 158-210. Figure 1 is a modified Figure 19.4, from Biochemical Protozoology, 214, Ed. G. Coombs, et al., Tayla and Francis, London (1991).

For fragment amplification, a number of oligonucleotides were tried without success until oligonucleotides specific for the *Plasmodium vinckei* cysteine proteinase, described in Example 2 were identified. These oligonucleotides were found to be suitable for and were therefore used to amplify a fragment of genomic DNA from Iowa isolate *Cryptosporidium parvum* oocysts.

The fragment was sequenced using methods described below and known in the art and found to encode a 459 bp portion of a cysteine proteinase gene seen in Figure 2, DNA residues 869-1326. The fragment was hybridized to an Iowa isolate genomic Southern blot which indicated that the cysteine proteinase was a single copy gene. Results are seen in Figure 5.

Figure 5 is a genomic Southern analysis of *Cryptosporidium* DNA using the cryptopain probe. In Figure 5, lane 1, the probe hybridizes to two Hind III fragments. These fragments are of approximate size 1.5 and 4 kb. In lane 2, the probe hybridizes with a Hae III fragment of 1.2 kb. In lane III the probe hybridizes to fragments of 1.2 and 4 kb of a Hind III/Hae III digest. In lane 4, the probe identifies fragments of 10 and 1 kb in an NsiI digest. In lane 5, the probe identifies a single band of 4 kb in an ScrII digest and in lane 6 it identifies fragments of 1.0, .5 and 4 kb in an NsiI/ScrII digests. The presence of 1 or 2 bands greater than the size of the probe in all digests indicates that the cysteine proteinase is a single copy gene.

The 459 bp Iowa fragment was then used to identify naturally infected neonatal calf (NINC) according to Infect.

Immun., 61:40 (1993) library clone which encoded the entire gene and 5' and 3' flanking regions. The sequence of this clone appears in Figure 2 and is identified as (SEQ ID NO: 1). The sequence of the open reading frame was determined.

5 The corresponding sequences of the NINC clone and the 459 bp sequence of the Iowa cysteine proteinase isolate are identical indicating that cryptopain is highly conserved in these isolates and that its function is essential for *Cryptosporidium*.

10 Sequences identified as SEQ ID NOS: 1-6 disclosed in this invention are new. These sequences represent nucleotides and amino acid sequences of *C. parvum* antigen. They were prepared according to methods described in Examples 1, 2 and 3.

15 SEQ ID NO: 1 is the DNA sequence of the *Cryptosporidium* cryptopain. The sequence (SEQ ID NO: 1) comprises 1663 base pairs and comprises 5' and 3' flanking sequences, pre, pro (SEQ ID NO: 2) and mature enzyme (SEQ ID NO: 3) sequences.

20 SEQ ID NO: 4 is the amino acid sequence of the cryptopain. The cryptopain contains 401 amino acids and contains pre and pro fragments (SEQ ID NO: 5), and mature enzyme (SEQ ID NO: 6).

25 Sequences 7-8 are known and correspond to cysteine proteinases isolated from other organisms, namely from *Carica* and *P. vinckei*. Homology between these and the current *C. parvum* cysteine proteinase is shown and described in Figures 1 and 4.

 Sequences identified as SEQ ID NOS: 9-12 are primer sequences.

30 Sequences SEQ ID NOS: 13-15 represent amino acid fragments of cryptopain.

 Sequence SEQ ID NO: 16 represents a 1206 fragment of cryptopain DNA.

C. Structure of the Cryptopain Gene and Its Encoded

Protein

The function of cryptopain is highly correlated with the structure of the protein which is determined by the corresponding sequence. In addition, regulation of the function is, at least in part, dependent upon the presence of the pro sequence.

Sequence identified as SEQ ID NO: 1 (Figure 2) is a DNA sequence of cryptopain. Sequence identified as SEQ ID NO: 4 (Figure 3) is its corresponding protein. Search of the Gene Bank and Swiss Protein Bank revealed that these sequences were highly homologous to cathepsin L- like sequences of various organisms as seen in Figure 4.

Figure 4 is an amino acid alignment showing marked amino acid similarities of cryptopain of *Cryptosporidium* (SEQ ID NO: 4) cysteine proteinase (papain) of *Carica* (SEQ ID NO: 7) and mature cysteine proteinase *Plasmodium vinckei* (SEQ ID NO: 8). In Figure 4, the mature enzyme of *P. vinckei* and the pre pro enzymes of cryptopain and papain (*Carica*) are lined up.

The active site cysteine shown at site 200 is embedded in a 7 amino acid fragment CGSCWAF (SEQ ID NO: 13) which is conserved in all three enzymes and was one of the sites chosen to make degenerate oligonucleotides primers listed in Figure 7A. There is not a high degree of conservation of sequence between the 3 enzymes around the active site histidine seen at 341. However, the conserved arginine at 392 is embedded in an amino acid fragment YWL/IVRNSW (SEQ ID NO: 14) which only differs by 1 amino acid in *P. vinckei* cysteine proteinase and cryptopain. This substitution of I and L was not engineered into the degeneracy of the *P. vinckei* oligonucleotide. Nonetheless, the degenerate oligonucleotide 782 containing sequence for VRNFW (SEQ ID NO: 15) and the active site cysteine oligonucleotide 781 were specific enough to amplify the 459 bp fragment. Unlike cryptopain, the *P. vinckei* has a

large insertion seen in amino acids 358-386 between the conserved cysteine and arginine that were the basis for nucleotide PCR of the 459 bp *C. parvum* fragment.

D. Production of Cryptopain Recombinant Protein

5 Recombinant cryptopain proteins are useful as antigens for preparing antibodies which will inactivate cysteine proteinase and provide antibody probes to detect the presence of the organism in the environmental and clinical diagnostic setting. Their recombinant production is therefore important.

10 Recombinant proteins of the invention were produced as described in Example 5. Generally, the 1203 bp cryptopain open reading frame (ORF) is engineered for in frame expression as a thioredoxin fusion protein in the Invitrogen vector pTrxFus, or any other suitable vector seen diagrammed in
15 Figure 8. This vector is used to create C-terminal fusions to *E. coli* thioredoxin. There is a multiple cloning site which allows in frame fusion of foreign protein with thioredoxin. Between the thioredoxin and the foreign protein there is an enterokinase cleavage site. Enterokinase treatment permits
20 the release of thioredoxin from the protein. pTrxFus DNA is digested with for example KpNI and XbaI and the intervening fragment is removed for example, by gel purification.

Primers 7B1 and 7B2 were used to amplify the pre pro enzyme sequence from Iowa *Cryptosporidium* DNA. The primer 7B1
25 has a KpNI site and the primer 7B2 has an XbaI site engineered into the 5' end of the oligonucleotides. These enzymes are used to digest the amplified DNA so that it could be inserted directionally and in frame into the KpNI/XbaI restriction digested pTrxFus. Then, the vector, such as pTrxFus,
30 containing the sequence for the pre pro enzyme, is used to transform competent *E. coli* cells. Ampicillin resistant transformants are then analyzed for plasmid DNA by restriction with KpNI-XbaI and by sequence for the presence, orientation

and reading frame of the gene. Clones containing the same gene are induced for expression of cryptopain and expression of the fusion protein, such as for example cryptopain-thioredoxin, at 57 kD, was analyzed by SDS-PAGE as seen in Figure 9, followed by immunoblot with antithioredoxin antibody. Conditions for optimal production of soluble protein in *E. coli* are assessed.

Results of the actual preparation of recombinant cryptopain using vector pTrxFus are seen in Figure 9. Figure 9 shows proteins harvested from a lysed cell culture, i.e., the soluble supernatant proteins. Lane 1 is wild type thioredoxin. Lanes 2, 3 and 4 are thioredoxin cryptopains harvested from cell culture at 2, 3 and 4 hours of growth of thioredoxin cryptopain. The pellet fraction showed no fusion protein indicating that the cryptopain-thioredoxin is wholly soluble. Growth was maximal at 3 hours and degradation products of Mr less than 57 were visible at 4 hours indicating that the optional time for harvesting culture was around 3 hours.

Fusion protein may be purified by osmotic shock or heat treatment of cell lysates to produce highly purified fusion protein. The fusion protein is advantageously cleaved with enterokinase at a cleavage site comprising 4 asparagine and 1 lysine sequence.

Production of cryptopain may be accomplished in multiple procaryotic or eukaryotic cells, including baculovirus, insect cells, yeast and mammalian cells. Cryptopain is purified by any suitable method known in the art, such as incorporation of histidine and purification by nickel chromatography, heat treatment of fluoredoxin fusion protein with subsequent harvesting of soluble protein.

I. Inhibition of Sporozoite Invasion

In order to determine whether the invasion of

Cryptosporidium sporozoites may be inhibited, active site inhibitors of cathepsin L-like cysteine proteinases were investigated.

Cryptosporidiosis infection is caused by the invasion of
5 cells with *Cryptosporidium*, typically *Cryptosporidium parvum*.
In order to provide prophylactic, therapeutic, diagnostic or
detection agents, it is necessary to determine what the
function of cryptopain in the process of cell invasion is, and
whether or not during the *Cryptosporidium* cell invasion
10 cryptopain acts at the surface of the sporozoites. For this
reason, studies were performed using known inhibitors to
determine entry of the sporozoites into the cells.

Because the sequence of cryptopain (SEQ ID NO: 4) had
high homology with other cysteine proteinases, and has an N-
15 terminal hydrophobic region, it was decided to determine if *C.*
parvum secreted cysteine proteinase. The biotin modification
of phe-ala-fluoromethylketone (BPAFMK) makes it unable to
enter intact cells. Therefore, the biotinylated phe-ala
fluoromethylketone was used to determine whether a cathepsin-L
20 like cysteine proteinase was active either at the surface of
the *Cryptosporidium* sporozoites or in the supernatant media
during invasion of Madin Darby canine kidney (MDCK) host cells
and whether it allows *C. parvum* to enter the cells. Results
are seen in Figure 10.

25 Figure 10A shows the % of sporozoites invasion as a
function of the concentration of three cysteine proteinase
inhibitors, namely inhibitors trans-epoxysuccinyl-L-
leucylamido-(4-guanidino) butane (E64), obtained from Sigma,
St. Louis, BPAFMK, obtained from Enzyme Systems, Dublin,
30 California and K-III, research drug of Arris Pharmaceutical,
South San Francisco, California. The inhibitors were
administered within the range of 10^{-3} to 10^1 (0.001 to 10 mM).

As seen in Figure 10, one hundred nM of all three

inhibitors decreased invasion by *Cryptosporidium* to 20-30% of untreated controls. The inhibition of invasion of sporozoites by BPAFMK (Figure 10) shows that cryptopain is involved in a proteolytic events which are necessary for invasion and intracellular development of *Cryptosporidium*. Results seen in Figure 10 therefore show that cryptopain is either localized at the surface of the *Cryptosporidium* sporozoites, is a part of the sporozoites membrane or is localized internally and is released during the invasion of the host cell.

Assessment of other studied cysteine proteinase inhibitors (E64 and K-III) which were not chemically modified to prevent entry into the cell indicate that there is more than one cathepsin-L-like cysteine proteinase inhibitor which will prevent invasion and intracellular development.

Although not listed here, it is to be understood that other cysteine proteinase inhibitors, as long as these inhibitors inhibit *Cryptosporidium* invasion, are intended to be within the scope of this invention. The examples of active site inhibitors are trans-epoxysuccinyl-L-leucylamido-(4-guanidino) butane (E64), fluoromethylketone, diazomethanes, vinyl sulfones and cystatins.

Another class of inhibitors derived from pro region of cryptopain and its derivatives change the active enzyme into a proenzyme.

As described above, the complete DNA and amino acids structures of cryptopain (SEQ ID NOs: 1 and 4) comprise pre, pro and mature enzyme (SEQ ID Nos: 2 and 5) sequences (SEQ ID NOs: 3 and 6), delineated in Figure 1 and in Figure 4. These pre, pro and mature regions or elements are identified on the basis of homology to previously discovered and investigated cysteine proteinases, seen in Figure 1, compared to *Cryptosporidium parvum* cryptopain. Biochemical Protozoology, (*supra*). The N-terminus of the cryptopain sequence contains

pre pro sequence (SEQ ID NO: 2). The cryptopain pre sequence is predicted by Kyto-Doolittle hydropathy plot seen in Figure 6 to contain a hydrophobic sequence. Such sequences typically target the protein to a membrane.

5 Inactive cysteine proteinases are called proenzymes. Proenzymes of cysteine proteinases consist of at least a pro amino acid sequence which interacts conformationally with the contiguous mature enzyme sequence to render it inactive until the pro sequence is cleaved releasing the active mature
10 enzyme. Recent evidence indicates that the pro sequences of cysteine proteinases are excellent specific inhibitors of their respective mature active enzymes (Protein Eng., 8:59 (1995)).

Thus, the pro sequence (SEQ ID NO: 5) of cryptopain is a
15 good candidate and may be produced by recombinant or synthetic means for use as a pharmacological agent to prevent *Cryptosporidium* infection and/or the consequences of infection.

20 III. Prophylaxis or Treatment via Passive or Active Immunization

For protection and treatment of human or animal subjects subjected to exposure to *Cryptosporidium*, or subjects already suffering from *Cryptosporidium* infection, both passive or active immunization using the cryptopain antigen is
25 appropriate.

Surface active enzymes with confirmed essential functions for the parasite infectivity, like cryptopain, are targets for passive or active immunization. Cryptopain binds to antibodies which specifically bind to epitopes of
30 *Cryptosporidium* which are recognized by B and T cells.

For prophylactic, therapeutic or diagnostic purposes, the proteins of the invention are produced in large amounts by inserting the *Cryptosporidium parvum* DNA, described above,

into an expression vector such as pGEX, pET-9d, pTrxFus or baculovirus obtained from Invitrogen. The thus constructed hybrid vector is then used to transform or transfect a host. The host cells carrying the hybrid vector are then grown in a nutrient medium to allow the production of the gene product.

A number of transfer vectors are available for the production of protein from both full length and partial cDNA and genomic clones. Fused or non-fused protein products, depending on the vector used, constitute up to 50% of the total protein produced in infected cells. The thus obtained recombinant proteins are frequently immunologically and functionally similar to the corresponding endogenous proteins.

The obtained polypeptide is purified by methods known in the art or described in Examples. The degree of purification varies depending on the use of the polypeptide. For use in eliciting polyclonal antibodies, the degree of purity may not need to be very high. However, as in some cases impurities may cause adverse reactions, purity of 90-95% is typically preferred and in some instances even required. For the preparation of a pharmaceutical composition, however, the degree of purity must be high, as is known in the art.

When in a therapeutic composition, the polypeptide is combined with appropriate pharmaceutically acceptable excipients adjuvants and used for the immunization of immunocompetent patients who are at risk for cryptosporidiosis either at the time of immunization or in the future.

This group includes, but is not restricted to, HIV positive individuals who are still able to respond to vaccination, animal workers, health care workers, day care center children and their caretakers, and children in the developing world.

A. Antibodies and Their Production

A polyclonal or monoclonal antibody to native or

recombinant protein of the invention are useful in diagnosing and detecting *Cryptosporidium* as well as for treatment by providing a protection against the *Cryptosporidium* infections.

Anti-*Cryptosporidium* polyclonal antibodies recognizing
5 the cloned polypeptide are preferred over a monoclonal antibody (MAb) because they recognize multiple epitopes on the target polypeptide.

According to the method of the current invention, large amounts of recombinant cryptopain are produced by scale up
10 processes in commercial plants which enables production of a corresponding large quantity of polyclonal antibodies/or of immunogen for active immunization. The antibodies to recombinant expressed protein can also be produced according to the invention using the standard method available for
15 production of the antibodies to native protein.

Cryptopain comprising epitopes of *Cryptosporidium* that is recognized by intact B and/or T cells is produced in large amounts as described above and in Examples, purified and used to detect or characterize anti-*Cryptosporidium parvum* antibody
20 in the body substances of populations at risk of prior or current cryptosporidial infection. Cryptopain is also used for immunization. Typical intramuscular immunization schedules are as follows.

Cryptopain plus equal volume complete pharmaceutically
25 acceptable adjuvants and excipients is used at the beginning of immunization. Antigen plus equal volume incomplete adjuvant is used at week 2. Antigen plus equal volume incomplete adjuvant at week 4.

In addition, antibodies to such antigens are obtained by
30 immunizing animals, such as rabbits or goats, with the polypeptide plus adjuvant, as described above.

The antibodies of the invention are also used to detect *Cryptosporidium* antigens in body substances, for example,

stools of populations at risk of cryptosporidial infection by, e.g., collecting stool samples (Manual of Clinical Microbiology, 1986, supra), mixing with Streather's solution 1:4, and incubating with antibody followed by addition of a
5 fluorescein conjugated second antibody. In alternative, colorimetric labeling which do not require special microscope equipment or other detection methods also suitable.

B. Biologically Derived or Recombinant
Anti-Cryptosporidium Vaccines

10 Vaccine is a biologically derived or recombinantly prepared agent useful for artificially acquired immunization in a host. The current invention describes a production and provides biologically derived and recombinant vaccines for active immunization of animals and humans against
15 cryptosporidiosis and for the preparation of passive immune products for treatment of the established infection.

The scope of the invention is, therefore, intended to include both biologically derived or recombinantly prepared vaccines based on the antigens of the invention.

20 A recombinant vaccine is produced by identifying the relevant antigen or antigens of *Cryptosporidium* species, cloning them and expressing them using suitable vectors. This approach yields immunogens which are reproducible in sufficiently large quantities to allow preparation of vaccine
25 for active immunization. Recombinant vaccines are useful for immunization of the potential *Cryptosporidium* host, such as for example for inoculation of cows, and to produce the host's own antibodies against *Cryptosporidium* infection.

30 Additionally, the recombinant vaccines may be used for production of passive immunotherapeutic agents. For example, when the cow is inoculated with the vaccine it begins to produce hyperimmune colostrum. Hyperimmune colostrum from the immunized cows is then purified to yield Ig for passive

immunotherapy of immunocompromised persons, primarily AIDS patients, children, etc.

These vaccines are also useful for widespread use in calves to provide a primary protection against *Cryptosporidium* infection. Providing the herd with anti-*Cryptosporidium* immunity decreases the risk for waterborne outbreaks of cryptosporidiosis in areas where the watershed includes dairy lands. This provides a secondary benefit to human residents of those areas.

In addition, DNA or RNA may be introduced into a host such that propagation and/or expression of the encoded protein occurs in the host utilizing a so called "foreign expression system".

Anti-*Cryptosporidium* vaccine of the invention contains a *Cryptosporidium* antigen identified by the invention, modified in such a way that it is incapable of producing the *Cryptosporidium* symptoms but at the same time it is capable of eliciting the production of specific protective antibodies against the disease when introduced in the body.

Protection from cryptosporidiosis appears to be due to mucosal immunity which, if absent in AIDS patients, is difficult to establish but, if present, may afford protection against clinical cryptosporidiosis as AIDS progresses.

Thus, the invention describes vaccines able to provide active B cell-immunity and potentially T cell immunity against cryptosporidiosis in normal persons, in persons at risk for AIDS or in otherwise immunocompromised patients.

C. DNA and RNA Vaccines

Recently, new approaches appeared which utilize so called DNA or RNA vaccines. These approaches are described in Science, 259:1745 (1993), hereby incorporated by reference.

DNA or RNA vaccines or native immunity are produced according to the methods described *Ibid.* Briefly, nucleic

acid vectors containing *Cryptosporidium* antigen DNA nucleic acid are injected, preferably intramuscularly to the host. The nucleic acid enters or is transmitted where it results in production of antigen. The antigen elicits immune responses in the form of specific anti-*Cryptosporidium* antigen antibody or cell mediated immune events. In this way, the host receives DNA or RNA and provides his/her own humoral immunity and/or cell mediated responses.

IV. Diagnostic/Detection Utility

An important part of this invention is a method of diagnosing *Cryptosporidium* infection or detection of *Cryptosporidium* in the tissue samples or in the environment.

The diagnostic method comprises contacting a body fluid or tissue with an anti-*Cryptosporidium* polyclonal or monoclonal antibody having specificity for the antigen of this invention or its fragments, or vice versa, and ability to detect any selective binding of the antibody to any antigenic *Cryptosporidium* proteins present in the body fluid, tissue or specimen or selective binding of the antigen to the anti-*Cryptosporidium* antibody.

The detection of the antibody-antigen complex in body specimens or environmental samples may be conducted by any method known in the art. The detection methods include solid phase, double antibody, sandwich double antibody, and triple antibody assays, including ELISA and the like. Also suitable are enzyme-linked immunoassays and radioactively labeled assays.

Examples of body specimens are stools and other liquid or solid body output or tissue samples obtained from a subject. Examples of body fluids are blood, serum, saliva, urine, and the like. Methods for the preparation of the body substance and the body fluid are standard in the art and are described, for example in Manual of Clinical Microbiology, Chapter 8,

"Collection, Handling and Processing of Specimens", 4th edition, Eds, Lennette, E.H., Balows, A., Hausler, W.J. and Shadorny, A.J., American Society for Microbiology, (1986)).

Diagnosis and detection methods also comprise contacting
5 the DNA and RNA of body fluid, tissue, specimen and
environmental sample with DNA and RNA of the invention or
fragments thereof and the amplification of this specific
interaction via PCR, branched chain nucleic acid technology
and other amplification technologies such that the presence of
10 *Cryptosporidium* DNA and/or RNA in the bodily fluid, tissue,
specimen or environmental sample may be detected. Agents
suitable for immunodiagnostic use are proteins comprising
epitopes of *Cryptosporidium parvum* that are recognized by
intact B and/or T cells. These proteins are produced as
15 described above, purified and used to detect or characterize
anti-*Cryptosporidium parvum* antibody in the body substances of
populations at risk of prior or current cryptosporidial
infection. In addition, antibodies to such proteins are
obtained by immunizing animals, such as cows, rabbits or
20 goats, or birds with the vaccine combined with an adjuvant.

Additionally, detections of *Cryptosporidium* may be made
by determining cryptopain activity in biological or
environmental samples by methods used and known in the art.

V. Immunotherapy and Prophylaxis

25 The immunotherapy of cryptosporidiosis in humans and
animals may be conducted by administration of the antibodies
of the invention to patients with cryptosporidiosis to
effectively reduce their symptomatology.

A method for immunotherapeutic treatment, retardation, or
30 inhibition of *Cryptosporidium* infection comprises
administering to a subject in need of such treatment an amount
of an anti-*Cryptosporidium* polyclonal or monoclonal antibody
prepared according to the invention, effective to provide

immunity against the invasion of *Cryptosporidium* or effective to inhibit the existing *Cryptosporidium* infection.

A method of prophylaxis of *Cryptosporidium* infection comprises administering to a subject in need of such treatment
5 a vaccine, as described above, comprising the protein or recombinant protein of this invention capable of endogenous development of inhibitory amount of anti-*Cryptosporidium parvum* antibodies.

Typical immunization is achieved by inoculation of the
10 animal, bird or human host with the antigen protein combined with adjuvant.

For passive immunotherapy when used to passively immunize *Cryptosporidium* infected hosts, the polypeptide is first combined with appropriate adjuvants and used for the
15 immunization of cows or other donor animals to produce antibodies which may be administered to patients with cryptosporidiosis infection, particularly to AIDS patients, and to other immunocompromised hosts. Monoclonal antibodies produced in animals, in humans "humanized" from animal sources
20 and produced through chimeric techniques and other derivative techniques may be used for passive immunotherapy.

When in a therapeutic composition, the antigen protein is combined with appropriate adjuvants and used for the immunization of immunocompetent patients who are at risk for
25 cryptosporidiosis either at the time of immunization or in the future. This group includes, but is not restricted to, HIV positive individuals who are still able to respond to vaccination, animal workers, health care workers, day care center children and their caretakers, and children in the
30 developing world.

VI. Qualitative and Quantitative Detection of
Cryptosporidium-Formulations and Kits

Formulations suitable for the administration of

polypeptides and antibodies such as those described herein are known in the art. Typically, other components stimulatory of immune response as well as fillers, coloring, and the like may be added, such as pharmaceutically acceptable excipient,
5 additives and adjuvants.

For qualitative and quantitative determination of the presence of the *Cryptosporidium* infection and environmental contamination, a kit for the diagnosis/detection of *Cryptosporidium* is used. The kit comprises the polyclonal
10 antibody or antigen of this invention and a means for detecting the complexing of the antibody with antigen. Another such kit comprises DNA/RNA of the invention for use in detecting complementary DNA/RNA of cryptopain. Another such kit comprises PCR primers for amplification of cryptopain
15 sequences and a method of identifying them.

The kit is utilized for the detection of endogenous antibodies/antigens/DNA/RNA produced by a subject that is afflicted with cryptosporidiosis and antigens/DNA/RNA present in the environmental samples. Even at the early stages where
20 the parasite is commencing invasion of a subject's cells, some amount of the *Cryptosporidium* antigen or the specific antibody may be detected in serum. The kit detects either the antigen with the polyclonal antibodies or the presence of the anti-*Cryptosporidium* antibody with the antigen. The
25 complexing immunoreaction is detected by staining, radiography, immunoprecipitation or by any other means used in the art and suitable for these purposes.

In addition to the above, the kits may also comprise a control compounds, anti-antibodies, protein A/G, and the like,
30 suitable for conducting the different assays referred to above.

The current invention provides an effective treatment and prophylaxis against the cryptosporidiosis infection and means

of detection of the parasite and diagnosis of infection.

The following examples describe procedures used to prepare antigens, antibodies, vaccines and kits of the invention. They are illustrative only and any modification using methods known in the art is intended to be included. The following examples are not to be considered in any way limiting.

EXAMPLE 1

Cryptosporidium parvum Parasites

10 This example describes protocol used for isolation of *Cryptosporidium parvum* parasites from which the *Cryptosporidium* antigen was prepared.

Oocysts of the Iowa isolate of *Cryptosporidium parvum* were passaged through neonatal calves, (Pat Madin Pathasan, Pleasant Hill Farms, Idaho). The passaged oocysts were purified and encysted for use in invasion assays. The detailed protocol for purification and encysting is described in Infect. Immun., 61:4079 (1993). The described protocol was used unmodified.

20 For the DNA experiments described herein DNA was purified from 1×10^9 *Cryptosporidium parvum* according to Mol. Biochem Parasitol, 50:105-114, (1992).

EXAMPLE 2

Preparation of a *C. parvum* Cysteine Proteinase DNA Probe

25 This example describes procedural used for preparation of the *C. parvum* cysteine proteinase DNA probe.

Cysteine proteinases share DNA homology in the regions coding for the active site amino acids involved in proteolysis, specifically conserved C, H and N residues. This was used in choosing an appropriate oligonucleotide pairs to amplify the genomic DNA from Iowa isolate. The most suitable oligonucleotides were found to be those modeled in *Plasmodium vinckei* cysteine proteinase sequences around the conserved

cysteine (C) and arginine (N) residues as indicated in Figure 4.

Degenerate oligonucleotides for the active C and N sites of the *Plasmodium vinckei* cysteine proteinase were used to amplify a 459 bp genomic DNA fragment from Iowa isolate DNA. In the degenerate oligonucleotides a "/" indicates that the base pair on either side of the "/" could be included at that location in a triplet encoding an amino acid. (I) indicates inosine which will pair in hybridization reactions in a permissive manner. The oligonucleotides were PC4(sense) consisting of AAA-GGA-TCC-TGC/T-GGI-A/TG/CI-TGC/T-TGG-GCI-TT (SEQ ID NO: 9) encoding a BamHI site and the DNA sequence for C-G-S-C-W-A-F (SEQ ID NO: 13) and PC3 (anti-sense) consisting of the DNA sequence

TTT-GAA-TTC-CCA-IG/CA/T-A/GTT-IC/TT/G-IAC/T-IAT-CCA-A/GTA (SEQ ID NO: 10) encoding an Eco RI site and the antisense for a protein sequence. The protein sequence in the sense direction is Y-W-I-V/I-K/R-N-S-W (SEQ ID NO: 14). The restriction sites were not required for the experiments described here.

As shown in Figure 4, these oligonucleotides represented a 100% match for the seven amino acid C-G-S-C-W-A-F (SEQ ID NO: 13) sequence of *C. parvum* cryptopain and a 100% match for the five amino acids V-R-N-S-W (SEQ ID NO: 15) within the eight amino acid sequence surrounding the conserved N. These matches were sufficient for PCR amplification purposes.

One hundred nanograms of Iowa isolate DNA was amplified using reagents from GeneAmp (Perkin-Elmer, Foster City, Ca) under the following conditions:

Initial denaturation was 94°C for 2 minutes followed by 30 cycles of 94°C for 20 seconds, 40°C for 40 seconds and 72°C for 1 minute.

The 459 bp amplification product was isolated, subcloned in the TA vector (TA Cloning kit, Invitrogen, San Diego, CA)

and sequenced using the di-dioxy technique (Stategene Sequenase II Kit).

Iowa isolate sequence so obtained was found to be homologous to cysteine proteinases of a wide variety of organisms. The sequences of papain and the cysteine proteinase of *P. vinckei* are shown in Figure 4.

EXAMPLE 3

Isolation, Sequencing and Analysis of a *C. parvum* Cysteine proteinase gene

10 This example describes isolation, sequencing and analysis of a *C. parvum* cysteine proteinase gene encoding *C. parvum* antigen.

The 459 bp amplification product obtained in Example 2, containing a portion of an Iowa isolate *C. parvum* cysteine proteinase gene was labeled with d-dATP³² using random primers and Klenow fragment. The labeled 459 bp probe was used to screen a NINC, (naturally infected neonatal calf) λ gt11 genomic expression library.

20 Three clones, designated E1.6, E4 and RCB1.2, were identified in the library and were purified to homogeneity. Two of them, E1.6 and E4, were subcloned in Bluescript for sequencing (Sequence II kit) and were found to contain the complete cryptopain sequence and 5' and 3' flanking sequences as determined by analysis of the open reading frames within the clones and Genbank Search using the deduced amino acid sequence.

The entire sequence of E1.6 is designated SEQ ID NO: 1 and includes flanking sequences 5' and 3'. The mature enzyme sequence is designated SEQ ID NO: 3.

30

EXAMPLE 4

Southern Hybridization

This example describes Southern hybridization method used to detect the gene of the invention in genomic DNA.

One mg of Iowa DNA was digested with the restriction enzymes, Hind III, Hae III, Nsi, Scr II and combinations thereof, according to conditions for use of each enzyme as provided by the manufacturer (Promega). Digested DNAs were
5 subjected to electrophoresis in 0.8% agarose gels in 1X TAE. The gel was blotted to a nylon membrane Hybond N+, obtained from Amersham per manufacturer's instructions.

Results are seen in Figure 5 which shows a generic Southern analysis using DNA cut and separated in this manner
10 (lane 1=Hind III, lane 2=HaeIII, lane 3=HindIII/HaeIII, lane 4=NsiI, lane 5=SrcII and lane 6=Nsi/SrcII). The 459 bp probe was labeled with ³²P-ATP and hybridized to the membrane.

EXAMPLE 5

Preparation of Recombinant Cryptopain

15 This example describes the preparation of recombinant cryptopain.

The primers 7B1 and 7B2 (Figure 7B) were synthesized at the Biomedical Research center, University of California, San Francisco. 7B1 is a sense oligonucleotide and is comprised of
20 a KpnI restriction enzyme recognition site at the 5' end followed by coding sequence for the 5' end of the pre pro cryptopain sequence. 7B2 is an anti-sense oligonucleotide and is comprised of an XbaI sequence at the 5' end followed by the antisense coding sequence of the 3' end of the
25 preprocryptopain sequence. When used as a pair of PCR amplification oligonucleotides, these oligonucleotides allowed the amplification from genomic *Cryptosporidium* DNA of the entire cryptopain gene with Kpn 1 and Xba I sequences at the 5' and 3' ends respectively.

30 The 7BI and 7B2 sequences were designed so that after YpnI and Xba I digestion of the amplification product, the resultant fragment could be introduced in a directional manner into pTrxFus which was cut with KpnI/XbaI. Amplified and

restricted DNA was visualized on a 0.8% agarose-1XTAE gel using ethidium bromide. The amplified and endonuclease restricted band was excised from the gel and purified using a glass bead technique (Gene-Clean).

5 pTrxFus was also digested with the enzymes KpnI and Xba I, enzymes uniquely present in the sequence in the poly linker (Figure 8), and the small intervening sequence was removed by gel purification as noted above. pTrxFus and preprocryptopain DNA, prepared in this manner, at 1:1 and 1:5 molar ratios were
10 ligated overnight at 14°C in the presence of ligation buffer and T4 DNA ligase at a concentration of 50-250 ng insert DNA/10 µl.

G1724 chemically competent cells were made as described by Xi-Lvitrogen. Three to five µl of ligation mixes and
15 control mixes were introduced into separate tubes of competent cells and the tubes were incubated on ice for 30 minutes. Tubes were incubated in a 42°C heating block for 90 seconds and placed on ice for 2 minutes. Eight hundred µl of room temperature of enriched tryptone containing broth medium was
20 added to each tube and the tube was incubated with shaking at 30°C for 60 minutes. Twenty-five and 100 µl of each transformation mix was plated on RMG-Ampicillin transformation plates and the plates were incubated at 30°C overnight.

Nitrocellulose membrane replicas of colonies were
25 prepared from the transformation plates, the adherent cells lysed in alkaline solution and the DNA fixed to the membranes. Nitrocellulose membranes were hybridized with probes to contain cryptopain DNA and following hybridization with a cryptopain, probe were colony purified. DNA was purified from
30 colonies and the identity of the foreign DNA verified by restriction analysis and sequence analysis.

Purified colonies were grown in 1 µl aliquots for analysis. Growth conditions were varied with respect to time

(2, 3, 4 hours) and the bacteria lysed for evaluation of soluble and insoluble proteins. Results are shown in Figure 9.

Figure 9 shows soluble proteins from 10 μ l of lysate at 2, 3, 4 hours in lanes 2, 3, and 4 on an immunoblot of an SDS-PAGE gel. Cryptopain fused to thioredoxin appears as a 57 kDa protein which is appropriate for the size of the fusion partner (12 kDa) and the size of preprocryptopain (35 kDa). Lane 1 is the thioredoxin control. All lanes are visualized with anti-thioredoxin antibody followed by chemiluminescent detection (Amersham). Yield, using this expression system, was maximal at 3 hours of bacterial growth and was estimated at 0.9 mg cryptopain-thioredoxin per 250 ml culture. Although the yield was very high in this system, purification after enterokinase removal of the fusion partner was less satisfactory.

EXAMPLE 6

Large Scale Purification of Recombinant Cryptopain

This example describes the purification procedure for cryptopain.

In order to provide large quantities of cryptopain purified from its fusion partner, thioredoxin, the KpnI/XbaI preprocryptopain DNA fragment of Example 5 was cloned into an improved vector known as pThio His (Invitrogen). The improvements of the invitrogen system were:

- 1) Metal binding sites were engineered into the sequence between the thioredoxin reading frame and the enterokinase recognition site facilitating large scale purification of the fusion protein over chromatography columns (Pro-bond, Invitrogen).

- 2) Growth of transformed bacteria (Top 10, Invitrogen) in the presence of more standard media.

- 3) Ability to cleave the foreign protein from the fusion

partner using enterokinase while the fusion protein was on the nickel column allowing a high degree of purification from the fusion partner.

Colonies were prepared as in Example 5 using Top 10 *E. coli*. Large scale group was accomplished, the bacteria harvested and lysed and the fusion protein collected by passage over Probond or other metal chelation columns. The columns were washed with normal saline and cryptopain was collected by passing dilute enterokinase over the column.

10

EXAMPLE 7

Inhibition of *Cryptosporidium* Invasion and Intracellular Development in MDCK cells with Inhibitors of Cathepsin-L Like Cysteine Proteinases

This example describes studies performed to detect inhibition of *Cryptosporidium* invasion and intracellular development *in vitro* using cathepsin-L-cysteine proteinase inhibitors.

Cryptosporidium oocysts of the Iowa isolate were encysted according to Example 1. To assess the effect of inhibitors E64, BPAFMK and KIII on sporozoite invasion, inhibitors were incubated with viable sporozoites for 30 minutes prior to addition to monolayers of MDCK cells as described in (J. Protozool., 386:556 (1991); and Infect. Immunol., 61:4079 (1993)).

20

Sporozoite invasion and intracellular development in MDCK cells was scored at 16 hours after fixation of MDCK cells in formalin and staining with Giemsa.

EXAMPLE 8

Detection of proteinase activity as a measure of viability of *Cryptosporidium* organisms

30

This example describes a method for detection of proteinase activity as a measure for viability of *Cryptosporidium* organism in environmental samples.

Cryptosporidium cannot be grown in culture *in vitro*. Available evidence indicates that acquisition of cryptosporidiosis from water, food and other environmental sites is a major source of disease spread. However, reliable
5 methods of determining whether living *Cryptosporidium* species are present in a sample have not been developed.

The invention provides a method assaying activity of proteins which have a short half-life. Proteinases which are tightly regulated with respect to activation, because
10 unrestricted activity would damage the integrity of the cell, represent one such type of proteins.

Highly specific active site inhibitors of cryptopain are used for evaluation of viability of *Cryptosporidium* organisms. A highly specific inhibitor of cryptopain, for example E64,
15 KIII or pre pro cryptopain protein is labeled with a radioactive, chemiluminescent, colorimetric or other tag. The tagged inhibitor is incubated with *Cryptosporidium* organisms/proteins from an environmental sample and the amount of tag bound/organism relative to positive and negative
20 control is ascertained. Number of organisms may be determined by flow cytometry.

EXAMPLE 9

Agents Suitable for Passive Immunotherapy

This example describes preparation of suitable agents for
25 passive immuno therapy.

Recombinant cryptopain described in Example 5, or a recombinant fragment of cryptopain with or without fusion protein are used to immunize animals such as cows, goats or rabbits. The antibody developed in the body of the animal is
30 purified from serum or milk as colostrum or used without purification for treatment of a *Cryptosporidium* infection of mucosal surfaces.

The antibody is delivered orally or through a tube and is

optionally mixed with agents or substances which delay or prevent the inactivation of antibody in the gastrointestinal tract.

EXAMPLE 10

5 Agents Suitable for Active Immunotherapy

This example illustrates agents derived from *C. parvum* suitable for active immunotherapy.

Recombinant cryptopain according to Example 5, or recombinant fragments of cryptopain with or without fusion
10 protein is used to immunize animals or humans in such a way that the animal or human develops antibody or cell mediated immune responses to *Cryptosporidium* which ameliorate or inhibit infection by *Cryptosporidium*.

EXAMPLE 11

15 Agents Suitable for Immunodiagnostic/Immunodetection Use

This example illustrates procedure for obtaining agents derived from *Cryptosporidium parvum* for suitable immunodiagnostic/immunodetection use.

Recombinant cryptopain or recombinant fragments of
20 cryptopain or antibodies (monoclonal, polyclonal or chimeric) raised to recombinant cryptopain or recombinant fragments of cryptopain are used to detect the corresponding antibody or antigen in a soluble or fixed assay.

Recombinant cryptopain is immobilized in wells and
25 utilized to detect the corresponding antibody from humans or animals by capture of the antibody and colorimetric or other detection method.

Correspondingly. antibodies to recombinant cryptopain are immobilized in wells and utilized to detect cryptopain in
30 secretions or feces or other bodily fluids or environmental samples. Both of these assays are also be performed in a soluble format.

EXAMPLE 12

Detection of MRNA as a Measure of Viability
of *Cryptosporidium* Organisms

This example illustrates detection of mRNA as a measure of viability of *Cryptosporidium* organisms.

5 The presence of mRNAs which have a short half-life was assayed on the basis of the fact that many mRNAs are destroyed within 2 minutes of production and the amount of intact MRNA in a cell provides a measure of the viability of an organism.

10 A probe for hybridization with the MRNA of the invention is prepared and labelled with radioactive, chemiluminescent, colorimetric or other tag. The tagged probe is incubated with *Cryptosporidium* organisms from an environmental sample and the amount of tag bound/cell relative to positive and negative controls is ascertained. Number of organisms is determined by
15 flow cytometry or any other suitable means.

EXAMPLE 13

Agents Suitable For Nucleotide Based Diagnosis/Detection

20 This example illustrates the procedure for obtaining agents derived from *C. parvum* for nucleotides based diagnosis and/or detection.

 Oligonucleotides or PCR amplification products using nucleotides derived from the cryptopain or the flanking DNA sequences is used to detect *Cryptosporidium* in human or animal samples or in the environment.

25 Oligonucleotides are used to amplify a *Cryptosporidium* fragment as described in the Examples above from the samples or from the environment and to detect its presence in either location. PCR amplification products or segments of DNA or RNA are used as probes to detect the presence in either
30 location in hybridization experiments. Hybridization is either as a Southern blot or as a dot blot. The hybridization signal is amplified by a variety of techniques including the branched chain technique.

PCR Detection

EXAMPLE 14Preparation of Anti-Cryptosporidium Vaccines

This example describes preparation of anti-*Cryptosporidium* vaccines using DNA, RNA or amino acid cryptopain sequences.

A vaccine for prevention and treatment of infections caused by protozoan *Cryptosporidium* species (*Cryptosporidium*) in humans and other mammals was developed by utilizing newly identified and isolated DNA and amino acid sequences of the *Cryptosporidium* pathogen designated cryptopain.

The antigen proteins used for preparation of vaccines correspond to cryptopain (SEQ ID NO: 4) which is identified by being a target of the polyclonal or monoclonal antibodies of the invention capable of preventing or ameliorating disease and preventing invasion and/or intracellular development in host cells.

A DNA or RNA vaccine for prevention and treatment of infections caused by protozoan *Cryptosporidium* species (*Cryptosporidium*) in humans and other mammals was developed by utilizing newly identified and isolated DNA (SEQ ID NOs: 1-3) and amino acid sequences of the *Cryptosporidium* pathogen designated cryptopain.

A hybrid vector comprising a DNA segment that encodes the protein antigen able to bind selectively and specifically to anti-*Cryptosporidium* antibodies operatively coupled to the vector was prepared and expressed as described in Example 5. This includes preparation of recombinant vaccines using the viral expression vector according to Example 5 outside of the host body but also includes preparation of DNA vaccines and procaryotic or eukaryotic host carrying the hybrid vector which may be introduced into the host vertebrate or a direct introduction of DNA or RNA into host cells generating the hosts own expression or translation of DNA or RNA to produce

proteins eliciting appropriate antibodies.

EXAMPLE 15

Preparation of Anti-Cryptosporidium parvum Vaccine

This example illustrates procedure for preparation of
5 anti-Cryptosporidium parvum vaccine of the invention and its
use.

Vaccines use of recombinant Cryptosporidium antigens
prepared according to Examples 5 and 14.

(1) Antigens

10 Preferably 10-200 μ g of recombinant antigen of the
invention, either alone or in combination is sued for
preparation of the vaccine.

(2) Adjuvant

Any one of a number of adjuvants designed to either:

15 (a) stimulate mucosal immunity; or

(b) target mucosal lymphoid tissue is sued for
preparation of the vaccine of the invention.

Examples of these adjuvants are: liposomes, saponins,
lectins, cholera toxin B subunit, E. coli labile toxin (LT) B
20 subunit, pluronic block copolymers, hydroxyapatite, plant
glucans, acetyl mannan (from Aloe Vera), aluminum hydroxide.

(3) Route of administration

Since the vaccine must stimulate mucosal immunity, it
preferably is delivered to a mucosal site.

25 Feasible routes of administration include: oral, nasal,
rectal, and vaginal. However, boosting may occur via another
route.

(4) Volume

The volume of the vaccine, while not particularly
30 important, should be in the range that would permit ease of
use. Preferred range would be about 0.5 ml-2.5 ml, including
adjuvant, per one vaccine dose.

(5) Boost schedule

Since this vaccine would be intended for immunocompromised individuals, one would expect the diminishing immune status to require a more aggressive boosting schedule than would otherwise be necessary.

5 The vaccine is administered to high risk patients initially when their immune status is reasonably good (i.e., CD4 count of >500). Booster schedules are typically given initially at 1 month after the primary immunization, and again every 3-4 months during progression of the immunodeficient state.

10

WHAT IS CLAIMED IS:

1. A purified native, synthetic or recombinant protein
5 having a sequence SEQ ID NO: 4 and its fragments and variants.

2. The protein of Claim 1 wherein the sequence SEQ ID
NO: 4 comprises sequences SEQ ID NO: 5 and SEQ ID NO: 6.

10 3. The protein of Claim 2 wherein the sequence is SEQ
ID NO: 6 corresponding to a mature enzyme.

4. The protein of Claim 3 wherein the sequence is SEQ
ID NO: 5 corresponding to a pre pro fragment.
15

5. The protein of Claim 4 which is an inhibitor of the
protein of Claim 3.

6. A DNA encoding a protein having a sequence SEQ ID
20 NO: 1 and its fragments and variants.

7. The DNA of Claim 6 comprising sequences SEQ ID NO:
2 and SEQ ID NO: 3.

25 8. An mRNA encoding a protein having a sequence SEQ ID
NO: 1.

9. An antibody specifically binding to an antigen
having sequence SEQ ID NO: 4.
30

10. The antibody of Claim 9 binding to a fragment of SEQ
ID NO: 4 said fragment having a sequence SEQ ID NO: 5.

11. The antibody of Claim 9 binding to a fragment of SEQ ID NO: 4 said fragment having a sequence SEQ ID NO: 6.

12. The antibody of Claim 9 which are monoclonal or polyclonal.

13. A natural, synthetic or recombinant vaccine comprising a protein having a sequence SEQ ID NO: 4 useful for active immunization of a host against *Cryptosporidium* infection and an appropriate pharmaceutically acceptable adjuvant, said vaccine adapted to immunize a subject against cryptosporidiosis so that after immunization infection with *Cryptosporidium* elicits from the subject an amount of anti-*Cryptosporidium* antibodies sufficient to retard, inhibit or counter the infection.

14. The vaccine of Claim 12 wherein the protein has a sequence SEQ ID NO: 6.

15. A natural, synthetic or recombinant DNA or RNA vaccine having a nucleotide sequence SEQ ID NO: 1, wherein said vaccine is capable to endogenous elicit development of anti-*Cryptosporidium* antibodies.

16. A method of treatment of *Cryptosporidium* infections of comprising administering to a subject in need of such treatment an inhibitory amount of anti-*Cryptosporidium* antibodies binding to a protein having a sequence SEQ ID NO: 4.

30

17. A method of prophylaxis of *Cryptosporidium* infection comprising administering to a subject in need of such prophylaxis an amount of a protein having a sequence SEQ ID

NO: 4 capable of binding to the anti-*Cryptosporidium* antibody, said amount sufficient to elicit production of anti-*Cryptosporidium* antibodies.

5 18. A method of diagnosing *Cryptosporidium* infection, comprising steps:

 (a) contacting a sample of a body specimen, fluid or tissue obtained from a subject with an anti-*Cryptosporidium* antibody having specificity for an antigen having a sequence

10 SEQ ID NO: 4; and

 (b) detecting a formation of complex of the antibody/antigen present in the body sample.

ABSTRACT

5 CRYPTOPAIN VACCINES, ANTIBODIES, PROTEINS, PEPTIDES,
 DNA AND RNA FOR PROPHYLAXIS, TREATMENT AND DIAGNOSIS AND
 FOR DETECTION OF *Cryptosporidium* SPECIES

10 Vaccines, antibodies, proteins, DNAs and RNAs for
 diagnosis, prophylaxis, treatment and detection of
 Cryptosporidium species or *Cryptosporidium* species infections.
 Cryptosporidium species antigen and DNAs and RNA encoding the
 Cryptosporidium antigen and fragments thereof and recombinant
 proteins or fusion proteins produced thereby. Methods for
15 diagnosis, prophylaxis, treatment and detection of
 Cryptosporidium species infections.

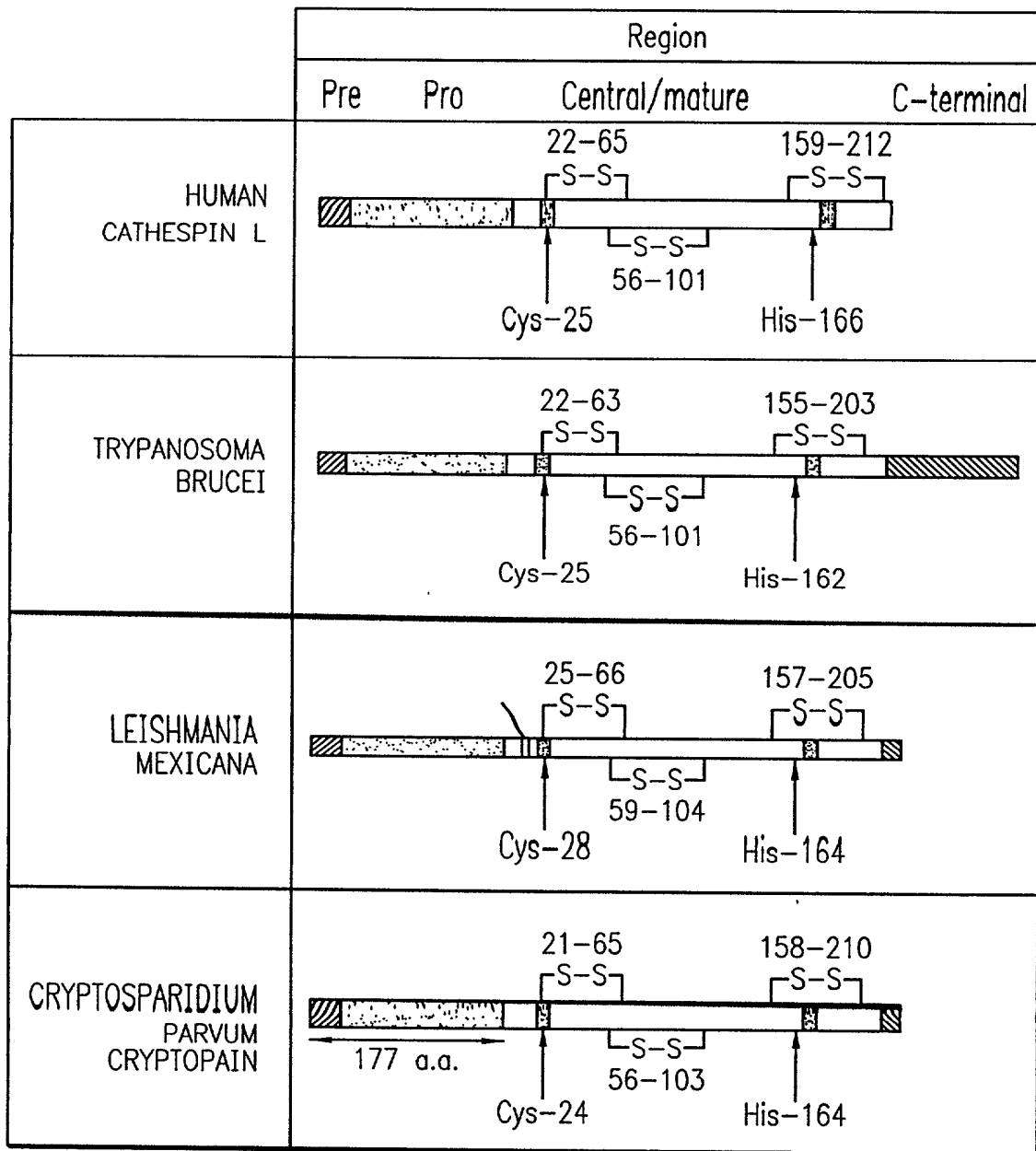


FIG. 1

1/1	31/11																
CAA AAC TTC CTA ATT TCT CAA TGT ATT ACT	AAT TAA TAG AAA GTT TGT TTT ATT TTC ATG																
gln asn phe leu ile ser gln cys ile thr	asn OCH AMB lys val cys phe ile phe met																
61/21	91/31																
TGG ATA AAT GAA TTA TTT TCT CTA TAC CGG	CAT TTG CAT GCA ATT TTG TAT GAC TAA AAT																
trp ile asn glu leu phe ser leu tyr arg	his leu his ala ile leu tyr asp OCH asn																
121/41	151/51																
GTA AAT AAT TAT TTG CAT GCA ATT ATG TGG	GCA TGT CAT AGT TTT TCA AGA ATA ATA ATA																
val asn asn tyr leu his ala ile met trp	ala cys his ser phe ser arg ile ile ile																
181/61	211/71																
AGA TGA CAT GAC AAG ATA TTC AAA AAA ATT	TGA TGA TTA TAT GTT GAA GTT AAT TGA ACT																
arg OPA his asp lys ile phe lys lys ile	OPA OPA leu tyr val glu val asn OPA thr																
241/81	271/91																
AAA AAG TAA TTA AGT AAA ATG GAC ATA GGA	AAC AAC GTG GAA GAA CAT CAG GAA TAT ATT																
lys lys OCH leu ser lys met asp ile gly	asn asn val glu glu his gln glu tyr ile																
301/101	331/111																
TCT GGA CCA TAC ATT GCA TTA ATT AAT GGC	ACT AAT CAA CAA AGG GAA CCG AAT AAA AAG																
ser gly pro tyr ile ala leu ile asn gly	thr asn gln gln arg glu pro asn lys lys																
361/121	391/131																
TTG AAA AAC ATA ATA ATT GCA ACG TTG ATT	GCA ATC TTT ATA GTT TTG GTT GTT ACT GTA																
leu lys asn ile ile ile ala thr leu ile	ala ile phe ile val leu val val thr val																
421/141	451/151																
TCT TTG TAT ATT ACT AAT AAC ACC AGT GAC	AAA ATT GAC GAT TTC GTA CCT GGT GAT TAT																
ser leu tyr ile thr asn asn thr ser asp	lys ile asp asp phe val pro gly asp tyr																
481/161	511/171																
GTT GAT CCA GCA ACT AGG GAG TAT AGA AAG	AGT TTT GAG GAG TTC AAA AAG AAA TAC CAC																
val asp pro ala thr arg glu tyr arg lys	ser phe glu glu phe lys lys lys tyr his																
541/181	571/191																
AAA GTA TAT AGC TCT ATG GAG GAG GAA AAT	CAA AGA TTT GAA ATT TAT AAG CAA AAT ATG																
lys val tyr ser ser met glu glu glu asn	gln arg phe glu ile tyr lys gln asn met																
601/201	631/211																
AAC TTT ATT AAA ACA ACA AAT AGC CAA GGA	TTC AGT TAT GTG TTA GAA ATG AAT GAA TTT																
asn phe ile lys thr thr asn ser gln gly	phe ser tyr val leu glu met asn glu phe																
661/221	691/231																
GGT GAT TTG TCG AAA GAA GAG TTT ATG GCA	AGA TTC ACA GGA TAT ATA AAA GAT TCC AAA																
gly asp leu ser lys glu glu phe met ala	arg phe thr gly tyr ile lys asp ser lys																
721/241	751/251																
GAT GAT GAA AGG GTA TTT AAG TCA AGT AGA	GTC TCA GCA AGC GAA TCA GAA GAG GAA TTT																
asp asp glu arg val phe lys ser ser arg	val ser ala ser glu ser glu glu glu phe																
781/261	811/271																
GTT CCC CCA AAT TCT ATT AAT TGG GTG GAA	GCT GGA TGC GTG AAC CCA ATA AGA AAT CAA																
val pro pro asn ser ile asn trp val glu	ala gly cys val asn pro ile arg asn gln																
841/281	871/291																
AAG AAT TGT GGG TCA TGT TGG GCT TTC TCT	GCT GTT GCA GCT TTG GAG GGA GCA ACG TGT																
lys asn cys gly ser cys trp ala phe ser	ala val ala ala leu glu gly ala thr cys																
901/301	931/311																
GCT CAA ACA AAC CGA GGA TTA CCA AGC TTG	AGT GAA CAG CAA TTT GTT GAT TGC AGT AAA																
ala gln thr asn arg gly leu pro ser leu	ser glu gln gln phe val asp cys ser lys																

FIG. 2-B

961/321

CAA AAT GGC AAC TTT GGA TGT GAT GGA GGA ACA ATG GGA TTG GCT TTT CAG TAT GCA ATT
gln asn gly asn phe gly cys asp gly gly thr met gly leu ala phe gln tyr ala ile
1021/341

AAG AAC AAA TAT TTA TGT ACT AAT GAT GAT TAC CCT TAC TTT GCT GAG GAA AAA ACA TGT
lys asn lys tyr leu cys thr asn asp asp tyr pro tyr phe ala glu glu lys thr cys
1081/361

ATG GAT TCA TTT TGC GAG AAT TAT ATA GAG ATT CCT GTA AAA GCC TAC AAA TAT GTA TTT
met asp ser phe cys glu asn tyr ile glu ile pro val lys ala tyr lys tyr val phe
1141/381

CCG AGA AAT ATT AAT GCA TTA AAG ACT GCT TTG GCT AAG TAT GGA CCA ATT TCA GTT GCA
pro arg asn ile asn ala leu lys thr ala leu ala lys tyr gly pro ile ser val ala
1201/401

ATT CAG GCC GAT CAA ACC CCT TTC CAG TTT TAT AAA AGT GGA GTA TTC GAT GCT CCT TGT
ile gln ala asp gln thr pro phe gln phe tyr lys ser gly val phe asp ala pro cys
1261/421

GGA ACC AAG GTT AAT CAT GGA GTT GTT CTA GTT GAA TAT GAT ATG GAT GAA GAT ACT AAT
gly thr lys val asn his gly val val leu val glu tyr asp met asp glu asp thr asn
1321/441

AAA GAA TAT TGG CTA GTA AGA AAT AGC TGG GGT GAA GCG TGG GGA GAG AAA GGA TAC ATC
lys glu tyr trp leu val arg asn ser trp gly glu ala trp gly glu lys gly tyr ile
1381/461

AAA CTA GCT CTT CAT TCT GGA AAG AAG GGA ACA TGT GGT ATA TTG GTT GAG CCA GTG TAT
lys leu ala leu his ser gly lys lys gly thr cys gly ile leu val glu pro val tyr
1441/481

CCA GTG AAT AAT CAA TCA ATA TAA GCA TTT CAG TGT TTG ACT AAG TAA TTC TAA TAT ATT
pro val ile asn gln ser ile OCH ala phe gln cys leu thr lys OCH phe OCH tyr ile
1501/501

TCA GCA TTC TCA GAG ATA ATT TTA GTT CAA ATG AAC AAT CTA TTC ATA TAT ATA AGC ATT
ser ala phe ser glu ile ile leu val gln met asn asn leu phe ile tyr ile ser ile
1561/521

CCA TAC TTA ATT ATT TAT TGA TTT TAA TAA AAT GTT TGG CTA AAG AAA GCA ATC AAG ATA
pro tyr leu ile ile tyr OPA phe OCH OCH asn val trp leu lys lys ala ile lys ile
1621/541

ATT TAT GGA CGT TCT ATT GTT CTT ACT TCA ATA ATA ATC CTT
ile tyr gly arg ser ile val leu thr ser ile ile ile leu

991/331

1051/351

1111/371

1171/391

1231/411

1291/431

1351/451

1411/471

1471/491

1531/511

1591/531

1651/551

FIG. 3-A

4/9

met	asp	ile	gly	asn	asn	val	glu	glu	his	gln	glu	tyr	ile	ser
1				5					10					15
gly	pro	tyr	ile	ala	leu	ile	asn	gly	thr	asn	gln	gln	arg	glu
				20					25					30
pro	asn	lys	lys	leu	lys	asn	ile	ile	ile	ala	thr	leu	ile	ala
				35					40					45
ile	phe	ile	val	leu	val	val	thr	val	ser	leu	tyr	ile	thr	asn
				50					55					60
asn	thr	ser	asp	lys	ile	asp	asp	phe	val	pro	gly	asp	tyr	val
				65					70					75
asp	pro	ala	thr	arg	glu	tyr	arg	lys	ser	phe	glu	glu	phe	lys
				80					85					90
lys	lys	tyr	his	lys	val	tyr	ser	ser	met	glu	glu	glu	asn	gln
				95					100					105
arg	phe	glu	ile	tyr	lys	gln	asn	met	asn	phe	ile	lys	thr	thr
				110					115					120
asn	ser	gln	gly	phe	ser	tyr	val	leu	glu	met	asn	glu	phe	gly
				125					130					135
asp	leu	ser	lys	glu	glu	phe	met	ala	arg	phe	thr	gly	tyr	ile
				140					145					150
lys	asp	ser	lys	asp	asp	glu	arg	val	phe	lys	ser	ser	arg	val
				155					160					165
ser	ala	ser	glu	ser	glu	glu	glu	phe	val	pro	pro	asn	ser	ile
				170					175					180
asn	trp	val	glu	ala	gly	cys	val	asn	pro	ile	arg	asn	gln	lys
				185					190					195
asn	cys	gly	ser	cys	trp	ala	phe	ser	ala	val	ala	ala	leu	glu
				200					205					210
gly	ala	thr	cys	ala	gln	thr	asn	arg	gly	leu	pro	ser	leu	ser
				215					220					225
glu	gln	gln	phe	val	asp	cys	ser	lys	gln	asn	gly	asn	phe	gly
				230					235					240
cys	asp	gly	gly	thr	met	gly	leu	ala	phe	gln	tyr	ala	ile	lys
				245					250					255
asn	lys	tyr	leu	cys	thr	asn	asp	asp	tyr	pro	tyr	phe	ala	glu
				260					265					270
glu	lys	thr	cys	met	asp	ser	phe	cys	glu	asn	tyr	ile	glu	ile
				275					280					285
pro	val	lys	ala	tyr	lys	tyr	val	phe	pro	arg	asn	ile	asn	ala
				290					295					300
leu	lys	thr	ala	leu	ala	lys	tyr	gly	pro	ile	ser	val	ala	ile
				305					310					315
gln	ala	asp	gln	thr	pro	phe	gln	phe	tyr	lys	ser	gly	val	phe
				320					325					330
asp	ala	pro	cys	gly	thr	lys	val	asn	his	gly	val	val	leu	val

FIG. 3-B

	335		340		345
glu tyr asp met	asp glu asp thr asn lys	glu tyr trp leu val			
	350		355		360
arg asn ser trp	gly glu ala trp gly glu lys gly tyr ile lys				
	365		370		375
leu ala leu his	ser gly lys lys gly thr cys gly ile leu val				
	380		385		390
glu pro val tyr	pro val ile asn gln ser ile				
	395		400 403		SEQ ID NO: 4

1000
 900
 800
 700
 600
 500
 400
 300
 200
 100
 0

```

60
Papain      . . . . . MAM IPSISKLLFV AICLFVYMG
Crypto pain MDIGNNVEEH QEISGPYIA LINGTNQORE PNKKLNIII ATLIAFIVL VVTVSLYITN
P.V., mature . . . . . NSQGFYSVLE
130
SFGDFS.I.VG YSQNDLTSTE RLIQLFESWM LKHNI YKNI DEKIYRFEIF KDNLYIDET NKKNNSYWLG
NT SDKIDDFV PGDYVDPATR EYRKSFEFEK KKYHKVYSSM EENQRFEIY KQNMNFIKT NSQGFYSVLE
. . . . .
200
LNVFADMSND EFKEKYTGSI AGNY. . . . TT TELSIEEVLN DGDVNIPEYV DWRQKGAVTP VKNQSGCGSC
MNEFGDLSKE EFMARFTGYI KDSKDDERVF KSSRVSASES EEEFVPPNSI NWVEAGCVNP IRNQKNCGSC
. . . . . FPDNR DYRSKFNFPL PKDQGNCGSC
CGSC
270
WAFSAVTIE GI IKIRTG.N LNEYSEQELL DCDR.. RSYG CNGGYPWSAL QLVAQY.GIH YRNTYPYEGV
WAFSAVAALE GATCAQTNRG LPSLSEQQFV DCSKQGNFG CDGGTMGLAF QYAIAKNKYLC TNDYDYPFAE
WAFAAIGNFE YLYVHTRHEM PISFSEQQMV DCST.. ENYG CDGGNPFYAF LYMINN.GVC LGDEYPYKGH
WAF
340
QRYC.RSREK GPYAAKTGCV RQVQPYNEGA LLYSIAN..Q PVSVVLEAAG KDFQLYRGGI FVGPCGNKVID
EKTCDMSFCE NYIEIPVKAY KYVFPNRINA LKTALAKY.G PISVAIQADQ TPFQFYKSGV FDPACGKTIV
EDFFCLNYRC SL LGRVHFIG DVKPNELI MA L..... NYVG PVTIANGA.S EDFVLYSGGV FDGECNPELN
410
HAVAAGVGP . . . . . NYILI KNSWGTGWG ENGYIRIKRG
HGVLVGVYDM DETNKE... YWLV RNSWGEAWG EKGYIKLALH
HSLVLLVGYGQ VKKSLAFEDS HSNVDSNL IK KYKENIKGDD DDDIYYWIV RNSWGNPWG EGGYIRIKRN
YWLV RNSW
433
TGNSYGVGCL YTSSFYPVKN ... SEQ ID NO : 7
.SGKKGTGCI LVEPVYPVIN QSI
KAGDDGFCGV GSDVFFPIY. ... SEQ ID NO : 8

```


FIG. 5

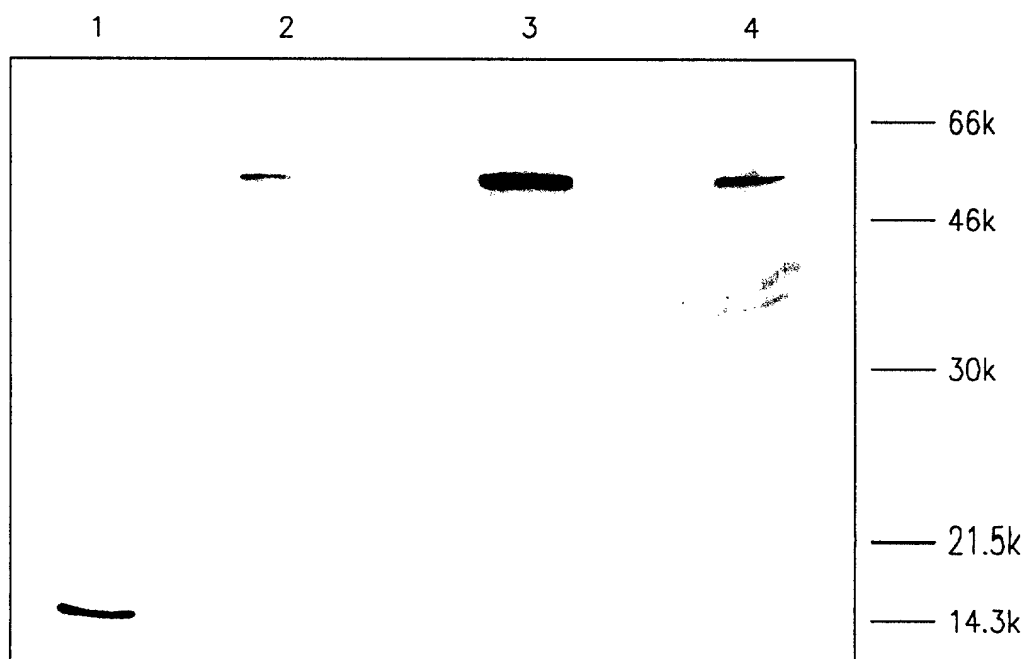
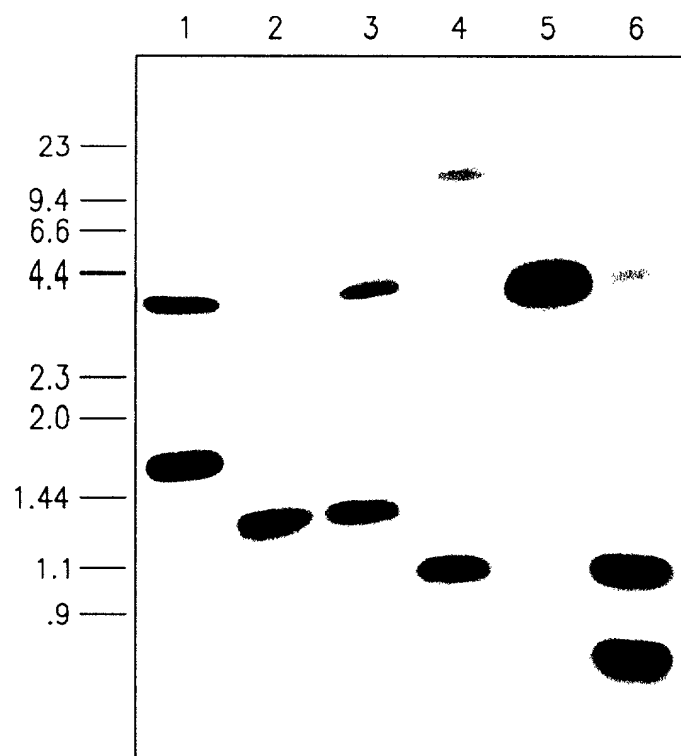


FIG. 6

FIG. 6

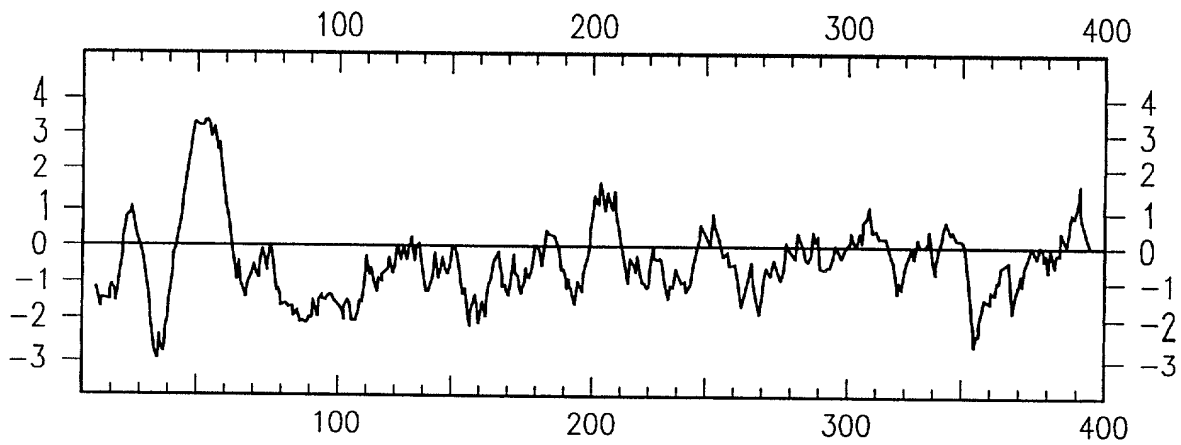


FIG. 7A

1. AAAGGATCCT GC/TGGIA/TG/CITG C/TTGGGCITT
 2. TTTGAATTCC CAIG/CA/TA/GTTIC/T T/GIAC/TIATCCA A/GTA

1. CCAGGTACCA TGGACATAGG AAAC
 2. CCCTCTAGAT GCTTATATTG ATTG

FIG. 7B

FIG. 8

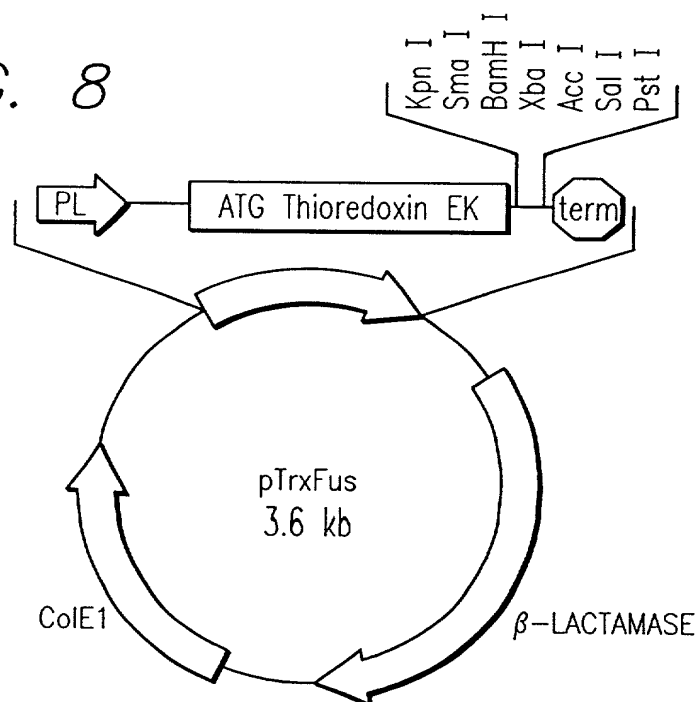


FIG. 10A

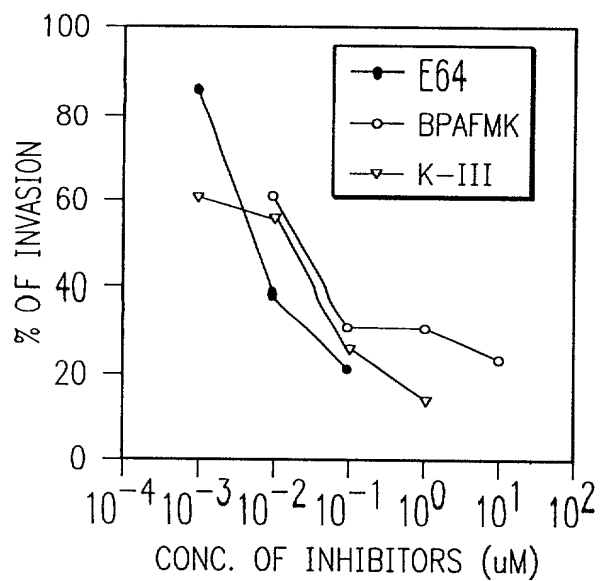


FIG. 10B

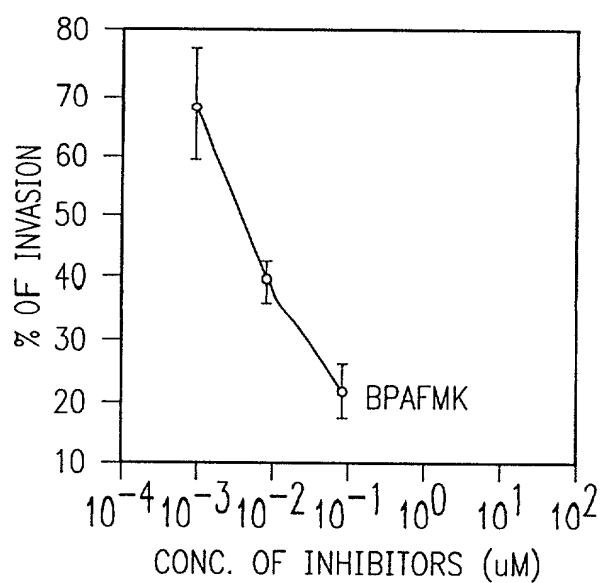


FIG. 10C

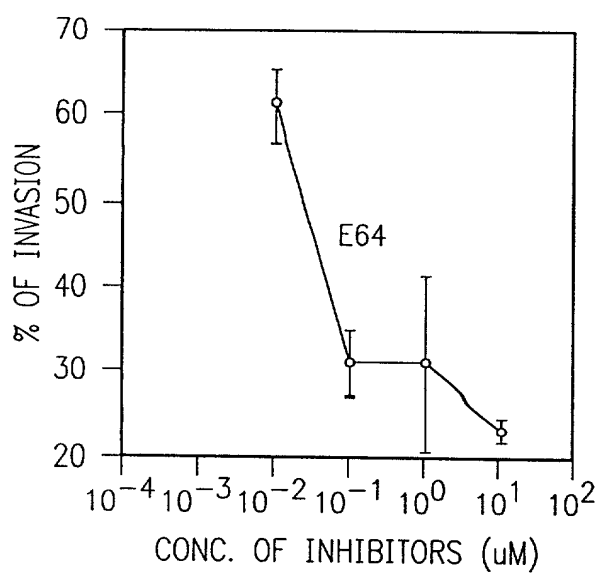
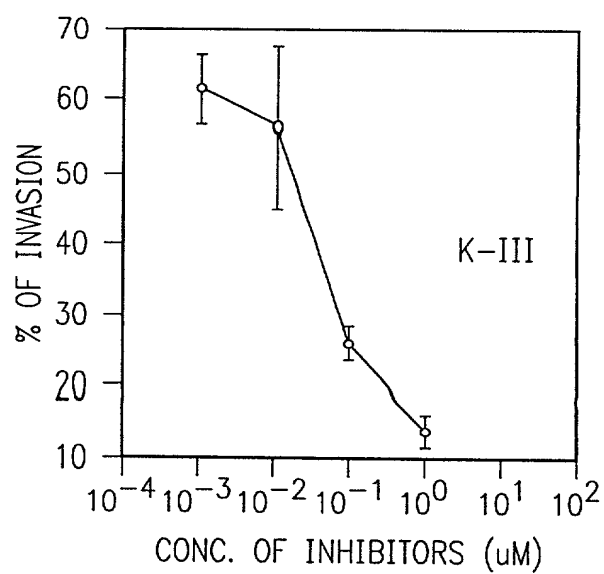


FIG. 10D



DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT OR CIP APPLICATION)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name, I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: CRYPTOPAIN VACCINES, ANTIBODIES, PROTEINS, PEPTIDES, DNA AND RNA FOR PROPHYLAXIS, TREATMENT AND DIAGNOSIS AND FOR DETECTION OF *Cryptosporidium* SPECIES

the specification of which: *(complete (a), (b) or (c) for type of application)*

REGULAR OR DESIGN APPLICATION

- (a) ☐ is attached hereto.
(b) ☒ was filed on March 27, 1997 as Application Serial No. 08/827,171.

PCT FILED APPLICATION ENTERING NATIONAL STAGE

- (c) ☐ was described and claimed in International Application No. _____ filed on _____ and as amended on _____

ACKNOWLEDGEMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose the information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations § 1.56(a).

☐ In compliance with this duty there is attached an information disclosure statement. 37 C.F.R. 1.97.

PRIORITY CLAIM

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

(complete (d) or (e))

- (d) ☒ no such applications have been filed.
(e) ☐ such applications have been filed as follows

**EARLIEST FOREIGN APPLICATION(S), IF ANY FILED WITHIN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION**

Country	Application No.	Date of filing (day, month, year)	Date of issue (day, month, year)	Priority Claimed
				<input type="checkbox"/> YES NO <input type="checkbox"/>
				<input type="checkbox"/> YES NO <input type="checkbox"/>
				<input type="checkbox"/> YES NO <input type="checkbox"/>

**ALL FOREIGN APPLICATION(S), IF ANY FILED MORE THAN 12 MONTHS
(6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION**

CONTINUATION-IN-PART

(complete this part only if this is a continuation-in-part application)

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

60/014,233 March 27, 1996 pending
(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

(Application Serial No.) (Filing Date) (Status) (patented, pending, abandoned)

POWER OF ATTORNEY

As a named inventor, I hereby appoint HANA VERNY, Registration No. 30,518, HOWARD M. PETERS, Registration No. 29,202, ALLSTON L. JONES, Registration No. 27,906, JANIS BIKSA, Registration No. 33,648, and CHARLES S. GUENZER, Registration No. 30,640 all of the address listed below, my principal attorney and agents, with full power of substitution and revocation, to appoint other principal and associate attorneys, to prosecute this application, and to transact all business in the Patent and Trademark Office connected therewith.

SEND CORRESPONDENCE TO:

Hana Verny
PETERS, VERNY, JONES & BIKŠA, L.L.P.
385 Sherman Avenue, Suite 6
Palo Alto, CA 94306-1840
Telephone No.: (415) 324-1677

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of sole or first inventor CAROLYN PETERSEN

Inventor's signature Carolyn Petersen

Date May 20, 1997 Country of Citizenship United States

Residence 82 FAIRLAWN DRIVE, BERKELEY, CALIFORNIA 94708

Post Office Address Same as above

Full Name of second joint inventor, if any JIN-XING HUANG

Inventor's signature Jin-Xing Huang

Date May 20, 1997 Country of Citizenship China

Residence 860 JAMESTOWN AVENUE, SAN FRANCISCO, CALIFORNIA 94124

Post Office Address Same as above

CHECK PROPER BOX(ES) FOR ANY ADDED PAGE(S) FORMING A PART OF THIS DECLARATION

- ☐ Signature for third and subsequent joint inventors. Number of pages added _____.
- ☐ Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. Number of pages added _____.
- ☐ Signature for inventor who refuses to sign or cannot be reached by person authorized under 37 C.F.R. 1.47. Number of pages added _____.

480-75.1

-1-

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Carolyn Petersen, et al.

Serial No.: 08/827,171

Filed: March 27, 1997

For: CRYPTOPAIN VACCINES, ANTIBODIES, PROTEINS,
PEPTIDES, DNA AND RNA FOR PROPHYLAXIS,
TREATMENT AND DIAGNOSIS AND FOR DETECTION
OF Cryptosporidium SPECIES




Box Sequence
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service as Express Mail Label No. EM061250792US in an envelope addressed to Assistant Commissioner for Patents, Washington, D.C. 20231 on July 28, 1998.



Hana Verna (Reg. No. 30,518)

DECLARATION SUBMISSION OF SEQUENCE LISTING UNDER
37 C.F.R. 1.821(c) AND 1.821(e)

Enclosed herewith are: (1) a paper entitled "Sequence Listing" (ten pages) for insertion after page 46 of the specification and before the claims; (2) a new computer readable form of the Sequence Listing, namely an ASCII text file named "480-75.1" on a DOS-formatted 3.5 inch, 1.44 Mb diskette; wherein the paper copy and the computer readable form are the same.

The Sequence Listing contains the identical sequences set forth in the application. No new matter is contained in the Sequence Listing.

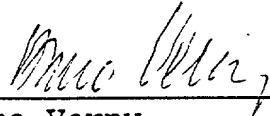
I hereby state that the content of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 CFR § 1.821(c) and (e), respectively, are the same.

I hereby state that the submission, filed in accordance with 37 CFR § 1.821 (g), herein does not include new matter.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Date: July 28, 1998



Hana VERNY
Reg. No. 30,518
Attorney of Record

PETERS, VERNY, JONES & BIKŠA, L.L.P.
385 Sherman Avenue, Suite 6
Palo Alto, CA 94306-1840
Telephone No.: (650) 324-1677
Attorney Docket No.: 480-75.1 (HV)
UC Case No. 96-279-2

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: CAROLYN PETERSEN
JIN-XING HUANG
- (ii) TITLE OF INVENTION: CRYPTOPAIN VACCINES, ANTIBODIES, PROTEINS,
PEPTIDES, DNA AND RNAs FOR PROPHYLAXIS,
TREATMENT, DIAGNOSIS AND
DETECTION OF
CRYPTOSPORIDIUM PARVUM
- (iii) NUMBER OF SEQUENCES: 16
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: PETERS, VERNY, JONES & BIKŠA
(B) STREET: 385 Sherman Avenue, Suite 6
(C) CITY: Palo Alto
(D) STATE: California
(E) COUNTRY: United States of America
(F) ZIP: 94306-1840
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette - 3.5 inch, 1.44 Kb storage
(B) COMPUTER: PC
(C) OPERATING SYSTEM: WINDOWS
(D) SOFTWARE: Wordperfect 6.0a WINDOWS
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/014,233
(B) FILING DATE: March 27, 1996
- (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Hana Verny
(B) REGISTRATION NUMBER: 30,518
(C) REFERENCE/DOCKET NUMBER: (HV)
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (415) 324-1677
(B) TELEFAX: (415) 324-1678

(2) INFORMATION FOR SEQ ID NO: 1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1663 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA
- (vi) ORIGINAL SOURCE:
(A) ORGANISM: *Cryptosporidium parvum*
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CAAACTTCC TAATTTCTCA ATGTATTACT AATTAATAGA AAGTTTGTTT TATTTTCATG	60
TGGATAAATG AATTATTTTC TCTATACCGG CATTTCATG CAATTTTGTA TGACTAAAAT	120
GTAAATAATT ATTTGCATGC AATTATGTGG GCATGTCATA GTTTTTC AAG AATAATAATA	180
AGATGACATG ACAAGATATT CAAAAAATT TGATGATTAT ATGTTGAAGT TAATTGAACT	240
AAAAAGTAAT TAAGTAAAAT GGACATAGGA AACAACGTGG AAGAACATCA GGAATATATT	300
TCTGGACCAT ACATTGCATT AATTAATGGC ACTAATCAAC AAAGGGAACC GAATAAAAAG	360

TTGAAAAACA	TAATAATTGC	AACGTTGATT	GCAATCTTTA	TAGTTTTGGT	TGTTACTGTA	420
TCTTTGTATA	TTACTAATAA	CACCAGTGAC	AAAATTGACG	ATTTTCGTACC	TGGTGATTAT	480
GTTGATCCAG	CAACTAGGGA	GTATAGAAAG	AGTTTGTAGG	AGTTCAAAAA	GAAATACCAC	540
AAAGTATATA	GCTCTATGGA	GGAGGAAAAAT	CAAAGATTGT	AAATTTATAA	GCAAAATATG	600
AACTTTATTA	AAACAACAAA	TAGCCAAGGA	TTCAGTTATG	TGTTAGAAAT	GAATGAATTT	660
GGTGATTTGT	CGAAAGAAGA	GTTTATGGCA	AGATTCACAG	GATATATAAA	AGATTCCAAA	720
GATGATGAAA	GGGTATTTAA	GTCAAGTAGA	GTCTCAGCAA	GCGAATCAGA	AGAGGAATTT	780
GTTCCCCCAA	ATTCTATTAA	TTGGGTGGAA	GCTGGATGCG	TGAACCCAAT	AAGAAATCAA	840
AAGAATTGTG	GGTCATGTTG	GGCTTTCTCT	GCTGTTGCAG	CTTTGCGAGG	ACCAACGTGT	900
GCTCAAACAA	ACCGAGGATT	ACCAAGCTTG	AGTGAACAGC	AATTTGTTGA	TTGCAGTAAA	960
CAAAATGGCA	ACTTTGGATG	TGATGGAGGA	ACAATGGGAT	TGGCTTTTCA	GTATGCAATT	1020
AAGAACAAAT	ATTTATGTAC	TAATGATGAT	TACCCTTACT	TTGCTGAGGA	AAAAACATGT	1080
ATGGATTTCAT	TTTGCAGAGAA	TTATATAGAG	ATTCCTGTAA	AAGCCTACAA	ATATGTATTT	1140
CCGAGAAATA	TTAATGCATT	AAAGACTGCT	TTGGCTAAGT	ATGGACCAAT	TTCAGTTGCA	1200
ATTCAGGCCG	ATCAAACCCC	TTTCCAGTTT	TATAAAAGTG	GAGTATTTCGA	TGCTCCTTGT	1260
GGAACCAAGG	TTAATCATGG	AGTTGTTCTA	GTTGAATATG	ATATGGATGA	AGATACTAAT	1320
AAAGAATATT	GGCTAGTAAG	AAATAGCTGG	GGTGAAGCGT	GGGGAGAGAA	AGGATACATC	1380
AAACTAGCTC	TTCATTCTGG	AAAGAAGGGA	ACATGTGGTA	TATTGGTTGA	GCCAGTGTAT	1440
CCAGTGATTA	ATCAATCAAT	ATAAGCATTT	CAGTGTTTGA	CTAAGTAATT	CTAATATATT	1500
TCAGCATTCT	CAGAGATAAT	TTTAGTTCAA	ATGAACAATC	TATTCATATA	TATAAGCATT	1560
CCATACTTAA	TTATTTATTG	ATTTTAATAA	AATGTTTGGC	TAAAGAAAAGC	AATCAAGATA	1620
ATTTATGGAC	GTTCTATTGT	TCTTACTTCA	ATAATAATCC	TTT		1663

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 534 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Cryptosporidium parvum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TTAAGTAAAA	TGGACATAGG	AAACAACGTG	GAAGAACATC	AGGAATATAT	TTCTGGACCA	60
TACATTGCAT	TAATTAATGG	CACTAATCAA	CAAAGGGAAC	CGAATAAAAA	GTTGAAAAAC	120
ATAATAATTG	CAACGTTGAT	TGCAATCTTT	ATAGTTTTGG	TTGTTACTGT	ATCTTTGTAT	180
ATTACTAATA	ACACCAGTGA	CAAAATTGAC	GATTTTCGTAC	CTGGTGATTA	TGTTGATCCA	240
GCAACTAGGG	AGTATAGAAA	GAGTTTTGAG	GAGTTCAAAA	AGAAATACCA	CAAAGTATAT	300
AGCTCTATGG	AGGAGGAAAA	TCAAAGATTT	GAAATTTATA	AGCAAAATAT	GAACCTTTATT	360
AAAACAACAA	ATAGCCAAGG	ATTCAGTTAT	GTGTTAGAAA	TGAATGAATT	TGGTGATTTG	420
TCGAAAGAAG	AGTTTATGGC	AAGATTACAA	CGATATATAA	AAGATTCCAA	AGATGATGAA	480
AGGGTATTTA	AGTCAAGTAG	AGTCTCAGCA	AGCGAATCAG	AAGAGGAATT	TGTT	534

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 678 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

Gly	Ala	Thr	Cys	Ala	Gln	Thr	Asn	Arg	Gly	Leu	Pro	Ser	Leu	Ser	215	220	225
Glu	Gln	Gln	Phe	Val	Asp	Cys	Ser	Lys	Gln	Asn	Gly	Asn	Phe	Gly	230	235	240
Cys	Asp	Gly	Gly	Thr	Met	Gly	Leu	Ala	Phe	Gln	Tyr	Ala	Ile	Lys	245	250	255
Asn	Lys	Tyr	Leu	Cys	Thr	Asn	Asp	Asp	Tyr	Pro	Tyr	Phe	Ala	Glu	260	265	270
Glu	Lys	Thr	Cys	Met	Asp	Ser	Phe	Cys	Glu	Asn	Tyr	Ile	Glu	Ile	275	280	285
Pro	Val	Lys	Ala	Tyr	Lys	Tyr	Val	Phe	Pro	Arg	Asn	Ile	Asn	Ala	290	295	300
Leu	Lys	Thr	Ala	Leu	Ala	Lys	Tyr	Gly	Pro	Ile	Ser	Val	Ala	Ile	305	310	315
Gln	Ala	Asp	Gln	Thr	Pro	Phe	Gln	Phe	Tyr	Lys	Ser	Gly	Val	Phe	320	325	330
Asp	Ala	Pro	Cys	Gly	Thr	Lys	Val	Asn	His	Gly	Val	Val	Leu	Val	335	340	345
Glu	Tyr	Asp	Met	Asp	Glu	Asp	Thr	Asn	Lys	Glu	Tyr	Trp	Leu	Val	350	355	360
Arg	Asn	Ser	trp	Gly	Glu	Ala	Trp	Gly	Glu	Lys	Gly	Tyr	Ile	Lys	365	370	375
Leu	Ala	Leu	His	Ser	Gly	Lys	Lys	Gly	Thr	Cys	Gly	Ile	Leu	Val	380	385	390
Glu	Pro	Val	Tyr	Pro	Val	Ile	Asn	Gln	Ser	Ile					395	400	403

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 175 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Cryptosporidium parvum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met	Asp	Ile	Gly	Asn	Asn	Val	Glu	Glu	His	Gln	Glu	Tyr	Ile	Ser	1	5	10	15
Gly	Pro	Tyr	Ile	Ala	Leu	Ile	Asn	Gly	Thr	Asn	Gln	Gln	Arg	Glu	20	25	30	
Pro	Asn	Lys	Lys	Leu	Lys	Asn	Ile	Ile	Ile	Ala	Thr	Leu	Ile	Ala	35	40	45	
Ile	Phe	Ile	Val	Leu	Val	Val	Thr	Val	Ser	Leu	Tyr	Ile	Thr	Asn	50	55	60	
Asn	Thr	Ser	Asp	Lys	Ile	Asp	Asp	Phe	Val	Pro	Gly	Asp	Tyr	Val	65	70	75	
Asp	Pro	Ala	Thr	Arg	Glu	Tyr	Arg	Lys	Ser	Phe	Glu	Glu	Phe	Lys	80	85	90	
Lys	Lys	Tyr	His	Lys	Val	Tyr	Ser	Ser	Met	Glu	Glu	Glu	Asn	Gln	95	100	105	
Arg	Phe	Glu	Ile	Tyr	Lys	Gln	Asn	Met	Asn	Phe	Ile	Lys	Thr	Thr	110	115	120	
Asn	Ser	Gln	Gly	Phe	Ser	Tyr	Val	Leu	Glu	Met	Asn	Glu	Phe	Gly				

	125	130	135
Asp Leu Ser Lys	Glu Glu Phe Met Ala	Arg Phe Thr Gly Tyr	Ile
	140	145	150
Lys Asp Ser Lys	Asp Asp Glu Arg Val	Phe Lys Ser Ser Arg	Val
	155	160	165
Ser Ala Ser Glu	Ser Glu Glu Glu Phe	Val	
	170	175	

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 226 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Cryptosporidium parvum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Pro	Pro	Asn	Ser	Ile	Asn	Trp	Val	Glu	Ala	Gly	Cys	Val	Asn	Pro	
1				5					10					15	
Ile	Arg	Asn	Gln	Lys	Asn	Cys	Gly	Ser	Cys	Trp	Ala	Phe	Ser	Ala	
				20					25					30	
Val	Ala	Ala	Leu	Glu	Gly	Ala	Thr	Cys	Ala	Gln	Thr	Asn	Arg	Gly	
				35					40					45	
Leu	Pro	Ser	Leu	Ser	Glu	Gln	gln	Phe	Val	Asp	Cys	Ser	Lys	Gln	
				50					55					60	
Asn	Gly	Asn	Phe	Gly	Cys	Asp	Gly	Gly	Thr	Met	Gly	Leu	Ala	Phe	
				65					70					75	
Gln	Tyr	Ala	Ile	Lys	Asn	Lys	Tyr	Leu	Cys	Thr	Asn	Asp	Asp	Tyr	
				80					85					90	
Pro	Tyr	Phe	Ala	Glu	Glu	Lys	Thr	Cys	Met	Asp	Ser	Phe	Cys	Glu	
				95					100					105	
Asn	Tyr	Ile	Glu	Ile	Pro	Val	Lys	Ala	Tyr	Lys	Tyr	Val	Phe	Pro	
				110					115					120	
Arg	Asn	Ile	Asn	Ala	Leu	Lys	Thr	Ala	Leu	Ala	Lys	Tyr	Gly	Pro	
				125					130					135	
Ile	Ser	Val	Ala	Ile	Gln	Ala	Asp	Gln	Thr	Pro	Phe	Gln	Phe	Tyr	
				140					145					150	
Lys	Ser	Gly	Val	Phe	Asp	Ala	Pro	Cys	Gly	Thr	Lys	Val	Asn	His	
				155					160					165	
Gly	Val	Val	Leu	Val	Glu	Tyr	Asp	Met	Asp	Glu	Asp	Thr	Asn	Lys	
				170					175					180	
Glu	Tyr	Trp	Leu	Val	Arg	Asn	Ser	Trp	Gly	Glu	Ala	Trp	Gly	Glu	
				185					190					195	
Lys	Gly	Tyr	Ile	Lys	Leu	Ala	Leu	His	Ser	Gly	Lys	Lys	Gly	Thr	
				200					205					210	
Cys	Gly	Ile	Leu	Val	Glu	Pro	Val	Tyr	Pro	Val	Ile	asn	Gln	Ser	
				215					220					225	
Ile															
226															

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 345 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Carica

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Met	Ala	Met	Ile	Pro	Ser	Ile	Ser	Lys	Leu	Leu	Phe	Val	Ala	Ile	5	10	15
Cys	Leu	Phe	Val	Tyr	Met	Gly	Leu	Ser	Phe	Gly	Asp	Phe	Ser	Ile	20	25	30
Val	Gly	Tyr	Ser	Gln	Asn	Asp	Leu	Thr	Ser	Thr	Glu	Arg	Leu	Ile	35	40	45
Gln	Leu	Phe	Glu	Ser	Trp	Met	Leu	Lys	His	Asn	Lys	Ile	Tyr	Lys	50	55	60
Asn	Ile	Asp	Glu	Lys	Ile	Tyr	Arg	Phe	Glu	Ile	Phe	Lys	Asp	Asn	65	70	75
Leu	Lys	Tyr	Ile	Asp	Glu	Thr	Asn	Lys	Lys	Asn	Asn	Ser	Tyr	Trp	80	85	90
Leu	Gly	Leu	Asn	Val	Phe	Ala	Asp	Met	Ser	Asn	Asp	Glu	Phe	Lys	95	100	105
Glu	Lys	Tyr	Thr	Gly	Ser	Ile	Ala	Gly	Asn	Tyr	Thr	Thr	Thr	Glu	110	115	120
Leu	Ser	Tyr	Glu	Glu	Val	Leu	Asn	Asp	Gly	Asp	Val	Asn	Ile	Pro	125	130	135
Glu	Tyr	Val	Asp	Trp	Arg	Gln	Lys	Gly	Ala	Val	Thr	Pro	Val	Lys	140	145	150
Asn	Gln	Gly	Ser	Cys	Gly	Ser	Cys	Trp	Ala	Phe	Ser	Ala	Val	Val	155	160	165
Thr	Ile	Glu	Gly	Ile	Ile	Lys	Ile	Arg	Thr	Gly	Asn	Leu	Asn	Glu	170	175	180
Tyr	Ser	Glu	Gln	Glu	Leu	Leu	Asp	Cys	Asp	Arg	Arg	Ser	Tyr	Gly	185	190	195
Cys	Asn	Gly	Gly	Tyr	Pro	Trp	Ser	Ala	Leu	Gln	Leu	Val	Ala	Gln	200	205	210
Tyr	Gly	Ile	His	Tyr	Arg	Asn	Thr	Tyr	Pro	Tyr	Glu	Gly	Val	Gln	215	220	225
Arg	Tyr	Cys	Arg	Ser	Arg	Glu	Lys	Gly	Pro	Tyr	Ala	Ala	Lys	Thr	230	235	240
Asp	Gly	Val	Arg	Gln	Val	Gln	Pro	Tyr	Asn	Glu	Gly	Ala	Leu	Leu	245	250	255
Tyr	Ser	Ile	Ala	Asn	Gln	Pro	Val	Ser	Val	Val	Leu	Glu	Ala	Ala	260	265	270
Gly	Lys	Asp	Phe	Gln	Leu	Tyr	Arg	Gly	Gly	Ile	Phe	Val	Gly	Pro	275	280	285
Cys	Gly	Asn	Lys	Val	Asp	His	Ala	Val	Ala	Ala	Val	Gly	Tyr	Gly	290	295	300
Pro	Asn	Tyr	Ile	Leu	Ile	Lys	Asn	Ser	Trp	Gly	Thr	Gly	Trp	Gly	305	310	315
Glu	Asn	Gly	Tyr	Ile	Arg	Ile	Lys	Arg	Gly	Thr	Gly	Asn	Ser	Tyr	320	325	330
Gly	Val	Cys	Gly	Leu	Tyr	Thr	Ser	Ser	Phe	Tyr	Pro	Val	Lys	Asn	335	340	345

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 244 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Plasmodium vinckei

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Phe Pro Asp Ser Arg Asp Tyr Arg Ser Lys Phe Asn Phe Leu Pro
5 10 15
Pro Lys Asp Gln Gly Asn Cys Gly Ser Cys trp Ala Phe Ala Ala
20 25 30
Ile Gly Asn Phe Glu Tyr Leu Tyr Val His Thr Arg His Glu Met
35 40 45
Pro Ile Ser Phe Ser Glu Gln Gln Met Val Asp Cys Ser Thr Glu
50 55 60
Asn Tyr Gly Cys Asp Gly Gly Asn Pro Phe Tyr Ala Phe Leu Tyr
65 70 75
Met Ile Asn Asn Gly Val Cys Leu Gly Asp Glu Tyr Pro Tyr Lys
80 85 90
Gly His Glu Asp Phe Phe Cys Leu Asn Tyr Arg Cys Ser Leu Leu
95 100 105
Gly Arg Val His Phe Ile Gly Asp Val Lys Pro Asn Glu Leu Ile
110 115 120
Met Ala Leu Asn Tyr Val Gly Pro Val Thr Ile Ala Val Gly Ala
125 130 135
Ser Glu Asp Phe Val Leu Tyr Ser Gly Gly Val Phe Asp Gly Glu
140 145 150
Cys Asn Pro Glu Leu Asn His Ser Val Leu Leu Val Gly Tyr Gly
155 160 165
Gln Val Lys Lys Ser Leu Ala Phe Glu Asp Ser His Ser Asn Val
170 175 180
Asp Ser Asn Leu Ile Lys Lys Tyr Lys Glu Asn Ile Lys Gly Asp
185 190 195
Asp Asp Asp Asp Ile Ile Tyr Tyr Trp Ile Val Arg Asn Ser Trp
200 205 210
Gly Pro Asn Trp Gly Glu Gly Gly Tyr Ile Arg Ile Lys Arg Asn
215 220 225
Lys Ala Gly Asp Asp Gly Phe Cys Gly Val Gly Ser Asp Val Phe
230 235 240
Phe Pro Ile Tyr
244

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: synthetic oligonucleotide

- (ix) FEATURE:
 (A) NAME/KEY:
 Y is C/T
 W is A/T
 S is C/G
 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

AAAGGATCCT GYGGNWSNTG YTGGGCNTT

29

- (2) INFORMATION FOR SEQ ID NO: 10:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 33 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic oligonucleotide
 (ix) FEATURE:
 (A) NAME/KEY:
 S is C/G
 K is G/T
 W is A/T
 R is A/G
 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION:
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TTTGAATTCC CANSWRTTNV KNAYNATCCA RTA

33

- (2) INFORMATION FOR SEQ ID NO: 11:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic oligonucleotide
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

CCAGGTACCA TGGACATAGG AAAC

24

- (2) INFORMATION FOR SEQ ID NO: 12:
 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: synthetic oligonucleotide

- (iv) ANTI- SENSE: YES
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

CCCTCTAGAT GCTTATATTG ATTG

24

- (2) INFORMATION FOR SEQ ID NO: 13:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 7 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Cys Gly Ser Cys Trp Ala Phe
 5 7

- (2) INFORMATION FOR SEQ ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 8 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptides
(ix) FEATURE:
 (A) NAME/KEY:
 Xaa at 4 is Val/Ile
 Xaa at 5 is Lys/Arg
 (B) LOCATION:
 (C) IDENTIFICATION METHOD:
 (D) OTHER INFORMATION:
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Tyr Trp Ile Xaa Xaa Asn Ser Trp
 5 8

- (2) INFORMATION FOR SEQ ID NO: 15:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 5 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
(ii) MOLECULE TYPE: peptide
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Val Arg Asn Ser Trp
 5

- (2) INFORMATION FOR SEQ ID NO: 16:
(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1203 base pairs

[illegible]

ATA

SEQUENCE LISTING

5

(1) GENERAL INFORMATION:

(i) APPLICANT:

CAROLYN PETERSEN
JIN-XING HUANG

10

(ii) TITLE OF INVENTION:

CRYPTOPAIN VACCINES,
ANTIBODIES, PROTEINS, PEPTIDES,
DNA AND RNAs FOR PROPHYLAXIS,
TREATMENT, DIAGNOSIS AND
DETECTION OF
CRYPTOSPORIDIUM PARVUM

15

(iii) NUMBER OF SEQUENCES: 15

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: PETERS, VERNY, JONES & BIKŠA
(B) STREET: 385 Sherman Avenue, Suite 6
(C) CITY: Palo Alto
(D) STATE: California
(E) COUNTRY: United States of America
(F) ZIP: 94306-1840

20

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette - 3.5 inch, 1.44 Kb storage
(B) COMPUTER: PC
(C) OPERATING SYSTEM: WINDOWS
(D) SOFTWARE: Wordperfect 6.0a WINDOWS

25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

30

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 60/014,233
(B) FILING DATE: March 27, 1996

35

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Hana Verny
(B) REGISTRATION NUMBER: 30,518
(C) REFERENCE/DOCKET NUMBER: (HV)

40

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (415) 324-1677
(B) TELEFAX: (415) 324-1678

45

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1663 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

50

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Cryptosporidium parvum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

55

CAAAACTTCC	TAATTTCTCA	ATGTATTACT	AATTAATAGA	AAGTTTGTTT	TATTTTCATG	60
TGGATAAATG	AATTATTTTC	TCTATACCGG	CATTTCATG	CAATTTTGTA	TGACTAAAAT	120
GTAAATAATT	ATTGTCATGC	AATTATGTGG	GCATGTCATA	GTTTTTCAAG	AATAATAATA	180
AGATGACATG	ACAAGATATT	CAAAAAAATT	TGATGATTAT	ATGTTGAAGT	TAATTGAACT	240
AAAAAGTAAT	TAAGTAAAT	GGACATAGGA	AACAACGTGG	AAGAACATCA	GGAATATATT	300
TCTGGACCAT	ACATTGCATT	AATTAATGGC	ACTAATCAAC	AAAGGGAACC	GAATAAAAAG	360
TTGAAAAACA	TAATAATTGC	AACGTTGATT	GCAATCTTTA	TAGTTTTGGT	TGTTACTGTA	420

60

	TCTTTGTATA	TTACTAATAA	CACCCAGTGAC	AAAATTGACG	ATTCGTACC	TGGTGATTAT	480
	GTTGATCCAG	CAACTAGGGA	GTATAGAAAG	AGTTTGGAGG	AGTTCAAAAA	GAAATACCAC	540
	AAAGTATATA	GCTCTATGGA	GGAGGAAAAAT	CAAAGATTTG	AAATTTATAA	GCAAAATATG	600
	AACTTTATTA	AAACAACAAA	TAGCCAAGGA	TTCAGTTATG	TGTTAGAAAT	GAATGAATTT	660
5	GCTGATTTGT	CGAAAGAAGA	GTTTATGGCA	AGATTCACAG	GATATATAAA	AGATTCCAAA	720
	GATGATGAAA	GGGTATTTAA	GTCAAGTAGA	GTCTCAGCAA	GCGAATCAGA	AGAGGAATTT	780
	GTTCCCCCAA	ATTCTATTAA	TTGGGTGGAA	GCTGGATGCG	TGAACCCAAT	AAGAAATCAA	840
	AAGAATTGTG	GGTCATGTTG	GGCTTTCTCT	GCTGTTGCAG	CTTTGGAGGG	AGCAACGTGT	900
	GCTCAAACAA	ACCGAGGATT	ACCAAGCTTG	AGTGAACAGC	AATTTGTTGA	TTGCAGTAAA	960
10	CAAAATGGCA	ACTTTGGATG	TGATGGAGGA	ACAATGGGAT	TGGCTTTTCA	GTATGCAATT	1020
	AAGAACAAAT	ATTTATGTAC	TAATGATGAT	TACCCTTACT	TTGCTGAGGA	AAAAACATGT	1080
	ATGGATTTCAT	TTTGCAGAGAA	TTATATAGAG	ATTCTGTAA	AAGCCTACAA	ATATGTATTT	1140
	CCGAGAAATA	TTAATGCATT	AAAGACTGCT	TTGGCTAAGT	ATGGACCAAT	TTCAGTTGCA	1200
	ATTCAGGCCG	ATCAAACCCC	TTTCCAGTTT	TATAAAAGTG	GAGTATTCTGA	TGCTCCTTGT	1260
15	GGAACCAAGG	TTAATCATGG	AGTTGTTCTA	GTTGAATATG	ATATGGATGA	AGATACTAAT	1320
	AAAGAATATT	GGCTAGTAAG	AAATAGCTGG	GGTGAAGCGT	GGCGACAGAA	AGCATACATC	1380
	AAACTAGCTC	TTCATTCTGG	AAAGAAGGGA	ACATGTGGTA	TATTGGTTGA	GCCAGTGTAT	1440
	CCAGTGATTA	ATCAATCAAT	ATAAGCATT	CAGTGTGTTGA	CTAAGTAATT	CTAATATATT	1500
	TCAGCATTCT	CAGAGATAAT	TTTAGTTCAA	ATGAACAATC	TATTCATATA	TATAAGCATT	1560
20	CCATACTTAA	TTATTTATTG	ATTTTAATAA	AATGTTTGGC	TAAAGAAAGC	AATCAAGATA	1620
	ATTTATGCAC	GTTCTATTGT	TCTTACTTCA	ATAATAATCC	TTT		1663

(2) INFORMATION FOR SEQ ID NO: 2:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 534 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Cryptosporidium parvum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

35	TTAAGTAAAA	TGGACATAGG	AAACAACGTG	GAAGAACATC	AGGAATATAT	TTCTGGACCA	60
	TACATTGCAT	TAATTAATGG	CACATAATCAA	CAAAGGGAAC	CGAATAAAAA	GTTGAAAAAC	120
	ATAATAATTG	CAACGTTGAT	TGCAATCTTT	ATAGTTTTGG	TTGTTACTGT	ATCTTTGTAT	180
	ATTACTAATA	ACACCAGTGA	CAAAATTGAC	GATTTTCGTAC	CTGGTGATTA	TGTTGATCCA	240
	GCAACTAGGG	AGTATAGAAA	GAGTTTTGAG	GAGTTCAAAA	AGAAATACCA	CAAAGTATAT	300
40	AGCTCTATGG	AGGAGGAAAA	TCAAAGATTT	GAAATTTATA	AGCAAAATAT	GAACCTTTATT	360
	AAAACAACAA	ATAGCCAAGG	ATTGAGTTAT	GTGTTAGAAA	TGAATGAATT	TGGTGATTTG	420
	TCGAAAGAAG	AGTTTATGGC	AAGATTCCAA	GGATATATAA	AAGATTCCAA	AGATGATGAA	480
	AGGGTATTTA	AGTCAAGTAG	AGTCTCAGCA	AGCGAATCAG	AAGAGGAATT	TGTT	534

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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 678 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Cryptosporidium parvum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

60	CCCCCAAATT	CTATTAATTG	GGTGGGAAGCT	GGATGCGTGA	ACCCAATAAG	AAATCAAAAG	60
	AATTGTGGGT	CATGTTGGGC	TTTCTCTGCT	GTTGCAGCTT	TGGAGGGAGC	AACGTGTGCT	120
	CAAAACAACC	GAGGATTACC	AAGCTTGAGT	GAACAGCAAT	TTGTTGATTG	CAGTAAACAA	180
	AATGGCAACT	TTGGATGTGA	TGGAGGAACA	ATGGGATTGG	CTTTTCAGTA	TGCAATTAAG	240
	AACAAATATT	TATGTACTAA	TGATGATTAC	CCTTACTTTG	CTGAGGAAAA	AACATGTATG	300

5 GATTCATTTT GCGAGAATTA TATAGAGATT CCTGTAAAAG CCTACAAATA TGTATTTCCG 360
 AGAAATATTA ATGCATTAAA GACTGCTTTG GCTAAGTATG GACCAATTTT AGTTGCAATT 420
 CAGGCCGATC AAACCCCTTT CCAGTTTTAT AAAAGTGGAG TATTCGATGC TCCTTGTGGA 480
 ACCAAGGTTA ATCATGGAGT TGTTCAGTT GAATATGATA TGGATGAAGA TACTAATAAA 540
 GAATATTGGC TAGTAAGAAA TAGCTGGGGT GAAGCGTGGG GAGAGAAAGG ATACATCAAA 600
 CTAGCTCTTC ATTCTGAAA GAAGGGAACA TGTGGTATAT TGGTTGAGCC AGTGTATCCA 660
 GTGATTAATC AATCAATA 678

(2) INFORMATION FOR SEQ ID NO: 4:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 401 amino acids

(B) TYPE: amino acids

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Cryptosporidium parvum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

20 met asp ile gly asn asn val glu glu his gln glu tyr ile ser
 1 5 10 15
 gly pro tyr ile ala leu ile asn gly thr asn gln gln arg glu
 20 25 30
 25 pro asn lys lys leu lys asn ile ile ala thr leu ile ala
 35 40 45
 ile phe ile val leu val val thr val ser leu tyr ile thr asn
 50 55 60
 asn thr ser asp lys ile asp asp phe val pro gly asp tyr val
 65 70 75
 30 asp pro ala thr arg glu tyr arg lys ser phe glu glu phe lys
 80 85 90
 lys lys tyr his lys val tyr ser ser met glu glu glu asn gln
 95 100 105
 arg phe glu ile tyr lys gln asn met asn phe ile lys thr thr
 110 115 120
 35 asn ser gln gly phe ser tyr val leu glu met asn glu phe gly
 125 130 135
 asp leu ser lys glu glu phe met ala arg phe thr gly tyr ile
 140 145 150
 40 lys asp ser lys asp asp glu arg val phe lys ser ser arg val
 155 160 165
 ser ala ser glu ser glu glu glu phe val pro pro asn ser ile
 170 175 180
 asn trp val glu ala gly cys val asn pro ile arg asn gln lys
 185 190 195
 45 asn cys gly ser cys trp ala phe ser ala val ala ala leu glu
 200 205 210
 gly ala thr cys ala gln thr asn arg gly leu pro ser leu ser
 215 220 225
 50 glu gln gln phe val asp cys ser lys gln asn gly asn phe gly
 230 235 240
 cys asp gly gly thr met gly leu ala phe gln tyr ala ile lys
 245 250 255
 55 asn lys tyr leu cys thr asn asp asp pro tyr phe ala glu
 260 265 270
 glu lys thr cys met asp ser phe cys glu asn tyr ile glu ile
 275 280 285
 pro val lys ala tyr lys tyr val phe pro arg asn ile asn ala
 290 295 300
 60 leu lys thr ala leu ala lys tyr gly pro ile ser val ala ile
 305 310 315
 gln ala asp gln thr pro phe gln phe tyr lys ser gly val phe

320 325 330
 asp ala pro cys gly thr lys val asn his gly val val leu val
 335 340 345
 5 glu tyr asp met asp glu asp thr asn lys glu tyr trp leu val
 350 355 360
 arg asn ser trp gly glu ala trp gly glu lys gly tyr ile lys
 365 370 375
 leu ala leu his ser gly lys lys gly thr cys gly ile leu val
 380 385 390
 10 glu pro val tyr pro val ile asn gln ser ile
 395 400 403

(2) INFORMATION FOR SEQ ID NO: 5:

- 15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 175 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: protein
 20 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Cryptosporidium parvum*
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

25 met asp ile gly asn asn val glu glu his gln glu tyr ile ser
 1 5 10 15
 gly pro tyr ile ala leu ile asn gly thr asn gln gln arg glu
 20 25 30
 pro asn lys lys leu lys asn ile ile ala thr leu ile ala
 35 40 45
 30 ile phe ile val leu val val thr val ser leu tyr ile thr asn
 50 55 60
 asn thr ser asp lys ile asp asp phe val pro gly asp tyr val
 65 70 75
 35 asp pro ala thr arg glu tyr arg lys ser phe glu glu phe lys
 80 85 90
 lys lys tyr his lys val tyr ser ser met glu glu glu asn gln
 95 100 105
 arg phe glu ile tyr lys gln asn met asn phe ile lys thr thr
 110 115 120
 40 asn ser gln gly phe ser tyr val leu glu met asn glu phe gly
 125 130 135
 asp leu ser lys glu glu phe met ala arg phe thr gly tyr ile
 140 145 150
 45 lys asp ser lys asp asp glu arg val phe lys ser ser arg val
 155 160 165
 ser ala ser glu ser glu glu glu phe val
 170 175

(2) INFORMATION FOR SEQ ID NO: 6:

- 50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 226 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 55 (ii) MOLECULE TYPE: protein
 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: *Cryptosporidium parvum*
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

60 pro pro asn ser ile asn trp val glu ala gly cys val asn pro
 1 5 10 15
 ile arg asn gln lys asn cys gly ser cys trp ala phe ser ala

20 25 30
 val ala ala leu glu gly ala thr cys ala gln thr asn arg gly
 35 40 45
 leu pro ser leu ser glu gln gln phe val asp cys ser lys gln
 5 50 55 60
 asn gly asn phe gly cys asp gly gly thr met gly leu ala phe
 65 70 75
 gln tyr ala ile lys asn lys tyr leu cys thr asn asp asp tyr
 80 85 90
 10 pro tyr phe ala glu glu lys thr cys met asp ser phe cys glu
 95 100 105
 asn tyr ile glu ile pro val lys ala tyr lys tyr val phe pro
 110 115 120
 15 arg asn ile asn ala leu lys thr ala leu ala lys tyr gly pro
 125 130 135
 ile ser val ala ile gln ala asp gln thr pro phe gln phe tyr
 140 145 150
 lys ser gly val phe asp ala pro cys gly thr lys val asn his
 155 160 165
 20 gly val val leu val glu tyr asp met asp glu asp thr asn lys
 170 175 180
 glu tyr trp leu val arg asn ser trp gly glu ala trp gly glu
 185 190 195
 25 lys gly tyr ile lys leu ala leu his ser gly lys lys gly thr
 200 205 210
 cys gly ile leu val glu pro val tyr pro val ile asn gln ser
 215 220 225
 ile
 226
 30

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- 35 (A) LENGTH: 345 amino acids
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) SOURCE ORIGIN:

- 40 (A) ORGANISM: Carica

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

met ala met ile pro ser ile ser lys leu leu phe val ala ile
 5 10 15
 cys leu phe val tyr met gly leu ser phe gly asp phe ser ile
 20 25 30
 45 val gly tyr ser gln asn asp leu thr ser thr glu arg leu ile
 35 40 45
 gln leu phe glu ser trp met leu lys his asn lys ile tyr lys
 50 55 60
 asn ile asp glu lys ile tyr arg phe glu ile phe lys asp asn
 65 70 75
 leu lys tyr ile asp glu thr asn lys lys asn asn ser tyr trp
 80 85 90
 leu gly leu asn val phe ala asp met ser asn asp glu phe lys
 95 100 105
 55 glu lys tyr thr gly ser ile ala gly asn tyr thr thr thr glu
 110 115 120
 leu ser tyr glu glu val leu asn asp gly asp val asn ile pro
 125 130 135
 60 glu tyr val asp trp arg gln lys gly ala val thr pro val lys
 140 145 150
 asn gln gly ser cys gly ser cys trp ala phe ser ala val val

[illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible][illegible]

CCCTCTAGAT GCTTATATTG ATTG

24

5

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

15 cys gly ser cys trp ala phe
5 7

20 (2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: peptides

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

tyr trp ile val/ile lys/arg asn ser trp
5 8

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(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids

(B) TYPE: amino acid

35 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

40 val arg asn ser trp
5

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1203 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

50 (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Cryptosporidium parvum*

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

55	ATGGACATAG	GAAACAACGT	GGAAGAACAT	CAGGAATATA	TTTCTGGACC	ATACATTGCA	60
	TTAATTAATG	GCACTAATCA	ACAAAGGGAA	CCGAATAAAA	AGTTGAAAAA	CATAATAATT	120
	GCAACGTTGA	TTGCAATCTT	TATAGTTTGT	GTTGTTACTG	TATCTTTGTA	TATTACTAAT	180
	AACACCACTG	ACAAAATTGA	CGATTTTCGT	CCTGGTGATT	ATGTTGATCC	AGCAACTAGG	240
	GAGTATAGAA	AGAGTTTGA	GGAGTTCAAA	AAGAAATACC	ACAAAGTATA	TAGCTCTATG	300
	GAGGAGGAAA	ATCAAAGATT	TGAAATTTAT	AAGCAAAATA	TGAACTTTAT	TAAAACAACA	360
60	AATAGCCAAG	GATTCAGTTA	TGTGTTAGAA	ATGAATGAAT	TTGGTGATTT	GTCGAAAGAA	420
	GAGTTTATGG	CAAGATTCAC	AGGATATATA	AAAGATTCCA	AAGATGATGA	AAGGGTATTT	480
	AAGTCAAGTA	GAGTCTCAGC	AAGCGAATCA	GAAGAGGAAT	TTGTTCCCCC	AAATTCTATT	540

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AATTGGGTGG	AAGCTGGATG	CGTGAACCCA	ATAAGAAATC	AAAAGAATTG	TGGGTCATGT	600
TGGGCTTTCT	CTGCTGTTGC	AGCTTTGGAG	GGAGCAACGT	GTGCTCAAAC	AAACCGAGGA	660
TTACCAAGCT	TGAGTGAACA	GCAATTTGTT	GATTGCAGTA	AACAAAATGG	CAACTTTGGA	720
TGTGATGGAG	GAACAATGGG	ATTGGCTTTT	CAGTATGCAA	TTAAGAACAA	ATATTTATGT	780
ACTAATGATG	ATTACCCTTA	CTTTGCTGAG	GAAAAACAT	GTATGGATTG	ATTTTGCAGG	840
AATTATATAG	AGATTCTGTG	AAAAGCTAC	AAATATGTAT	TTCCGAGAAA	TATTAATGCA	900
TTAAAGACTG	CTTTGGCTAA	GTATGGACCA	ATTTTCAGTTG	CAATTTCAGG	CGATCAAACC	960
CCTTTCCAGT	TTTATAAAAG	TGGAGTATTC	GATGCTCCTT	GTGGAACCAA	GGTTAATCAT	1020
GGAGTTGTTC	TAGTTGAATA	TGATATGGAT	GAAGATACTA	ATAAAGAATA	TTGGCTAGTA	1080
AGAAATAGCT	GGGGTGAAGC	GTGGGGAGAG	AAAGGATACA	TCAAACATAGC	TCTTCATTCT	1140
GGAAAGAAGG	GAACATGTGG	TATATTGGTT	GAGCCAGTGT	ATCCAGTGAT	TACTCAATCA	1200
ATA						1203

ATA